CROSS-FOSTERING FOR THE CONSERVATION OF RAT-KANGAROOS (MARSUPIALIA: POTOROIDAE)

by

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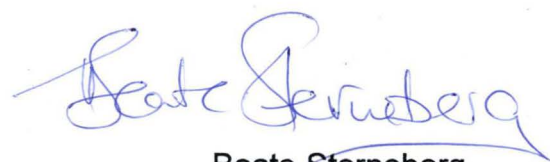

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02. March 2005

Beate Sterneberg

It is not a given
that it will be better
because it is different,

but if it is to get better
it will have to be different.

Translated from *Georg Christof Lichtenberg*

Abstract

Cross-fostering is an assisted reproductive method that has been used to improve the productivity of endangered marsupial fauna. Pouch young from endangered species are transferred to the pouch of related common species, allowing the donor female to produce further offspring without going through the entire lactation cycle.

In this project I tested the applicability of cross-fostering in rat-kangaroos (family Potoroidae) using the Tasmanian bettong (*Bettongia gaimardi*) and the Long-nosed potoroo (*Potorous tridactylus*) as model species. The effect of a 'transfer age difference' (TAD) between transferred young was investigated to determine the most beneficial time for pouch young transfer: optimising both the mother's reproductive rate and young's survival to independence.

Thirty two young were transferred, 18 intra-species and 14 inter-species. The transfers were carried out at various stages of pouch life (1 to 11 weeks) with TADs ranging from 0 to 3 weeks. Nutritional analyses were undertaken on 460 milk samples. Growth measurements and development data were obtained for 110 bettong and 46 potoroo young. The effect of cross-fostering on species-specific behaviour patterns was investigated using both video-recordings and direct observations.

Twenty one young survived and 19 subsequently bred successfully. Milk composition and production rates did not appear to be affected by transfers. Comparisons of growth rates between cross-fostered and either fostered or untransferred young showed an advantage for cross-fostered potoroos and a disadvantage for cross-fostered bettong. These were most pronounced during the period between pouch vacation and weaning. During asynchronous intra-species transfers the younger of the two transferees grew faster than untransferred bettong young. The magnitude of both these effects increased with TAD up to an age difference of three weeks when growth related problems appeared.

Although cross-fostered young were influenced by transfer mothers' behaviour, they developed normal species-specific behaviour. Difficulties in reuniting cross-fostered young with their own species were probably a consequence of different levels of sociability in the two species. At maturity, all surviving transfer young were successful in both mate recognition and production of offspring.

Growth and development of the transfer young are affected by the design of the transfer procedure. The combination of species, temperament and experience of the transfer mother; the age of young at transfer and the TAD all affect the outcome. The accessibility of the young in the pouch enables progress monitoring and, where required, early intervention for repeated transfers and/or hand-rearing. Recommendations for the management of transfer animals in captivity are provided.

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Chapter 1: General Introduction

The activities of the increasing global human population has resulted in reduction and fragmentation of animal habitat as well as a reduction in animal populations, leading to worldwide extinction of many species of animals (Bainbridge & Jabbour 1998) and plants (Johnston *et al.* 1999). Australia holds the record for biodiversity loss by suffering the highest rate of mammal extinction in the world (Renfree 1995). A substantial decline of the Macropodoidea (kangaroo group) followed European settlement of Australia and although determining causes for decline retrospectively is considered difficult, effects of land clearing, modification of vegetation by sheep and introduction of predators (foxes and cats) appear to be linked to the decline (Calaby & Grigg 1989).

Conservation efforts can be divided into *in situ* techniques, which require the protection of habitat (as well as the species living within it) (Soulé 1992), and *ex situ* techniques, which preserve and amplify a population of an endangered species outside its natural habitat (Caughley & Sinclair 1994). Although *in situ* or *on site* conservation is considered to be the better strategy for conserving biodiversity by enabling populations to adapt through natural evolutionary processes (Mazur 2001), it can not be applied to every species due to an increase in human disturbance (Primack 1995). *Ex situ* strategies secured the survival of animal species such as the Père David's Deer or Milu (*Elaphurus davidianus*) and the Przewalski horse (*Equus przewalski*) (Tudge 1993) as well as plant species e.g. *Franklinia alatamaha* (family Theaceae), which are already extinct in the wild (Primack 1995).

For endangered species, *ex situ* propagation offers advantages of allowing intense population management, reduction of possible extinction through stochastic influences (Caughley & Sinclair 1994) and protection against unexpected loss in the wild (e.g. disease or fire) (Johnston *et al.* 1999). Artificial breeding techniques also enable researchers to salvage and manage genetic material, assess fertility and screen for pathogens (Tribe *et al.* 1994).

Substantial research has been conducted over the last 20 years to determine the potential applications of reproductive technology for maintaining biodiversity (Johnston *et al.* 1999). Assisted reproductive techniques can be divided into three categories: the first set of techniques prepares the animal for the optimal reproductive state (oestrus synchronization and superovulation), the second set allows the transfer of genetic material between individuals (artificial insemination (AI), embryo transfer (ET), in vitro fertilization (IVF) and related techniques) while the third set builds a frozen genetic resource bank for possible selective use based on genetic analysis (Bainbridge & Jabbour 1998). Assisted reproductive techniques have been applied to a whole range of eutherian species including domestic and non-domestic bovids, cervids, felids and mustelids (summarised in Bainbridge & Jabbour 1998) as well as marsupial species (Tribe *et al.* 1994, Taggart *et al.* 1997, Mate *et al.* 1998 and Johnston *et al.* 1999).

The marsupial young is born at a very immature stage (weight at birth usually less than 0.01% of the mother's weight), climbs from the birth canal to the pouch opening unaided and completes most of its growth and development during lactation, while it remains fully accessible in the pouch for study (Tyndale-Biscoe & Janssens 1988). Since the focus of this thesis is pouch young transfer the subsequent discussions are mostly confined to marsupial literature given the fact that most transfers have been performed with macropod species.

Interspecies pouch young transfer is a non-invasive technique akin to embryo transfer, which takes advantage of the young's accessibility in the pouch. It utilises the unique mode of reproduction in marsupials by freeing the donor female from her young and therefore the burden of lactation and allowing her to return to oestrus and mate again, while the young is transferred into the pouch of a recipient female (Johnston *et al.* 1999). Merchant and Sharman (1966) showed that pouch young of various ages could be removed from the teat without injury and aided in re-attaching to the teat of a transfer mother of the same or different species. Further pouch young transfer were used to investigate growth rates (Clark 1968), rearing by transfer mothers of different species

(Johnson 1981), control of pouch vacation (Rose 1986), maternal behaviour (unpublished data in Russell 1989), release of embryonic diapause (Smith 1989), improvement of reproductive rate (unpublished data in Taggart *et al.* 1997), establishment of a captive colony (Smith 1998) and regulation of milk composition and production linked with pouch young development (Trott *et al.* 2003).

The above pouch young transfers were performed at different times of pouch life, but only little consideration was given to the possible impact of transfer age difference (between the transfer young and the young originally inhabiting the pouch) on growth and development of young. Clark (1968) stressed the importance of transfer age difference between young in regard to changes in milk composition for both intra- and inter-specific transfers and associated growth rates in young.

Compared to the relative consistent composition of eutherian milk, the composition of marsupial milk changes dramatically throughout lactation within a species, but remains relatively uniform between species (Green & Merchant 1988). Tyndale-Biscoe and Janssens (1988) identified three main phases and one subdivision in the marsupial lactation cycle (as opposed to two stages for eutherian species): phase one, during pregnancy (mammary gland develops capacity for milk synthesis, equivalent to lactogenesis stage one in eutherians); phase two, after parturition (young climbs unaided into the pouch and attaches to a teat, which begins the lactation cycle in the associated mammary gland while unsucked mammary glands regress; early phase two, young is continually attached; late phase two, young becomes physiologically mature) and the final phase three, production of lipid-rich milk (equivalent to lactogenesis stage two in eutherians).

The milk composition changes according to the needs of the young – an alteration, which Renfree (1983) refers to as 'tailor-made' for each stage of lactation. Merchant and Sharman (1966), Clark (1968) and Johnson (1981) provided evidence for altered growth rates (retarded as well as accelerated growth) in

transfer young due to inappropriate milk composition for the age and development of the young. Trott *et al.* (2003) investigated the hypothesis that the sucking pattern of the pouch young determined the mammary development. The authors found that the lactating female regulates milk composition and milk production rate and that both determine growth rate and development of pouch young irrespective of the transfer young's age.

Throughout lactation the young is nourished and cared for by its mother, but Walser (1977) states that lactation is of no significance without the appropriate behaviour of mother and young, which allows suckling to take place. Various aspects of mother-young interactions have been investigated for marsupial species including early behavioural development (Russell 1973), vocal communication (Baker & Croft 1993) and play behaviour (Byers 1999). Only little and inconsistent information is available on the behaviour of transfer mother-young dyads following parturition (Merchant and Sharman 1966, Johnson 1981).

Most of the available literature on pouch young transfers is inadequate, since researchers concentrated on particular topics, e.g. changes in milk composition and associated survival and growth rates in transfer young, rather than choosing a more holistic approach. Behavioural data needs to be included in the analysis to verify the transfer young's ability to exhibit species-specific behaviour as well as its reproductive success once mature. The objectives of this thesis are therefore defined as follows:

1) Investigation of the applicability of intra-species (fostering) and inter-species pouch young transfer (cross-fostering) to the Tasmanian bettong (*Bettongia gaimardi*) and the Long-nosed potoroo (*Potorous tridactylus*) within a captive colony.

2) Determination of the impact of the young's age at time of transfer as well as transfer age difference between transfer young on survival, growth and development of young as well as their reproductive success when matured.

3) Can the transfer age difference be used for adjusting growth and development in young if normal growth rates between the species differ?

4) Does pouch young transfer have an impact on mate recognition and species-specific behaviour patterns?

5) Recommendations for the management of transfer mother-young dyads in captivity

The results of this thesis could be useful for maximising the survival rate and successful rearing of transfer pouch young while providing animals for captive colonies, research and release into the wild as well as recommendations for their captive management.

Chapter 2: Study Animals and General Methods

2.1 Study Animals

The Tasmanian bettong (*Bettongia gaimardi*) and the Long-nosed potoroo (*Potorous tridactylus*) represent the study animals in this thesis. They are members of the subfamily Potoroinae, which, together with the subfamily Hypsiprymnodontinae, are members of the family Potoroidae. They are related to the larger kangaroos, wallabies and tree-kangaroos (family Macropodidae). Both families form the superfamily Macropodoidea.

Fig.2.1: A) Tasmanian bettong mother-young pair. B) Long-nosed potoroo breeding pair (male on the right, female in the middle) with subadult son.

Although extinct on the mainland of Australia, the Tasmanian bettong is still found in Tasmania. Its status is considered 'lower risk (near threatened)' (Burbidge 1999). Land clearing, excessive grazing of stock and implementation of 1080 poison for wallaby control on private land pose current threats (Maxwell *et al.* 1996) as well as the recent introduction of foxes (also applicable for Long-nosed potoroos). The Tasmanian bettong is patchily distributed throughout eastern Tasmania with dry sclerophyll forests on poor soil and an open understorey as the preferred habitat (Watts 1993). The forest trees form a symbiotic relationship with underground fungi, which produce the main part of the bettong's diet (fruiting bodies of the fungi), but seeds, roots and bulbs are also

consumed (Rose & Johnson 1995). The Long-nosed potoroo occurs in south-eastern Australia (status: vulnerable, Burbidge 1999) and Tasmania (status: Lower Risk [least concern], Maxwell *et al.* 1996). It can be found in most forest types and heath land with a minimum annual rainfall of 760 mm and prefers a thick ground cover on light and sandy soil (Johnston 1995). Their main food source also consists of underground fungi, but invertebrates and tubers are consumed as well.

The Tasmanian bettong and Long-nosed potoroo appear not to compete if present in the same area (Rose & Johnson 1995). Although both are considered solitary species, trapping records indicated the aggregation of individual Long-nosed potoroos in small groups (Johnston 1995). Both species are nocturnal, but the Long-nosed potoroo may also be seen at dusk (Seebeck *et al.* 1989). They construct nests of dry grass and bark to rest in during the day. Their tail is sufficiently prehensile for carrying nesting material (Strahan 1995). The nests of the Long-nosed potoroos are lacking a complex structure compared to the building style of the Tasmanian bettong (Seebeck *et al.* 1989).

Rose (1989) reviewed the reproductive patterns in the Potoroidae. Tasmanian Bettongs had a shorter gestation period (21.3 days) and pouch life duration (106 days) than Long-nosed potoroos (gestation length; 38 days, pouch life: 125 days). The annual fecundity was higher in Tasmanian bettongs (3.5, Long-nosed potoroos: 2.5). Although young appear to be weaned at the same time (24 weeks), female young of the Tasmanian bettong matured earlier (9 months) than of the Long-nosed potoroo (12 months). While the Tasmanian bettong is classified as a continuous breeder (Rose and Johnson 1995), the breeding pattern of Long-nosed potoroo is not well defined (Bryant 1989). Long-nosed potoroos show peaks in breeding during the end of winter and in early spring as well as late summer (Johnston 1995). Although the litter size in both species is one (Rose 1989), the rare occurrence of twinning has been reported for the genus *Bettongia* (Gansloßer 1988, no information on captive status) and free-living Long-nosed potoroos (Johnson & Buchmann 1993).

2.2 General Methods

2.2.1 Trapping

At the beginning of this project the University's rat-kangaroo colony consisted of 33 Tasmanian bettongs. The trapping of Long-nosed potoroos was conducted between December 1999 and March 2000 (Parks and Wildlife Service, Tasmania, Scientific Collecting Permit No: FA 99148, Animal Ethics Committee Approval No. 98072). Twelve potoroos (seven females and five males) were caught in different bush land locations close to the University using mascot traps. The traps were positioned within or close to established animal runways or potential nesting sites. They were placed under thick grass bushes or wooden logs, which acted not only as camouflage but also as shelter from the elements. A piece of apple covered in peanut butter was used as bait in the back of the trap. The soil at the entrance of the trap was disturbed to imitate digging signs of another animal in an attempt to make it more attractive to approach the trap and explore it. Traps were set in the late afternoon and checked at dawn the next morning.

Traps needed to be approached with care, since rat-kangaroos show severe signs of stress, injuring themselves and/or young (if present) while attempting to escape (this study). Once a potoroo was found in the trap, a dark hessian bag was tightly placed around the entrance prior to opening the door. Usually animals would prefer the dark environment of the bag to being exposed in the wire trap. On occasions when stressed animals froze their movements completely, the trap was tilted slightly. A blow of air onto the fur would usually be sufficient for the animal to move. The bag was then closed quickly to prevent the animal from climbing out while removing the trap. The animal's sex and reproductive status was determined (2.2.9.1 Physical restraint). If the particular animal was suitable for the project, it remained in the bag while being carried back to the university for marking with a microchip and subsequent introduction to the captive colony.

If a decision was made against collection of the animal, the bag was placed loosely opened on the ground facing away from the trapping site. The bag was

supervised quietly until the animal chose to leave it. All traps were left closed at the trapping site during the day to prevent the accidental trapping of unwanted species (e.g. birds, lizards) and were reset again in late afternoon.

2.2.2 Identification

All adults received a microchip (Destron Fearing Corporation, manufactured for AEIDS PTY LTD, microchip type: TX1750L), which was inserted subdermally between the shoulder blades using a sterile injector applicator. There were several occasions when younger animals lost their microchip. Therefore the insertion was delayed until offspring reached sub-adulthood or adulthood unless *'reliable'* identification was required earlier. Animals with unique body conditions (e.g. one eye, absent tail) did not receive a microchip. More information on visual identification can be found under 6.2.1 Visual Animal Identification.

2.2.3 Quarantine

Animals were presented to a veterinarian if they sustained injuries or displayed any other forms of disease, parasite infestation or signs of distress. This was particularly important when animals first came into captivity to prevent diseases from being introduced to the colony. Spare cages were made available if animals needed to be separated. Strict guidelines were followed for the maintenance of the captive colony (NHMRC 1997, Australian code of practice for the care and use of animals for scientific purposes). If an animal did not adjust to captivity, veterinarian advice was obtained and in the case of a ≥10% body weight loss, an adult (including pouch young if present) would be released at the site of capture while a pouch young would be hand-reared.

2.2.4 Hygiene

Inappropriate husbandry and/or stress are two of the main factors inducing disease in captive held animals (Austin 1997, White 1997, Walraven 1999, Woods 1999). Therefore strict hygiene is essential in disease management and prevention. Food and water bowls were cleaned meticulously with antibacterial dishwashing liquid at least three times a week. They were rinsed several times with clean water to ensure no remaining residue. Faeces and soiled food were

removed from the cage on a daily basis. Contents and cleanliness of food and water bowls as well as sufficient supply of nesting material were checked every day. All bedding and nesting material were replaced at least once a month unless required earlier (wet winter months). The material was recycled afterwards for garden purposes outside of the animal enclosures. While all bedding material was removed, cages with a concrete floor were scrubbed with disinfectant and subsequently hosed thoroughly. Outside areas were raked and all contaminated topsoil was removed.

2.2.5 Enclosure types and environmental enrichment

Initially, a small group of animals had to be housed indoors using an artificial day/night cycle. However, the housing arrangement was considered inadequate for this project since insufficient sunlight may cause Vitamin D deficiency (Staker 2001, Williams & Williams 1999). Eventually all animals were housed outdoors in an animal compound exposed to a natural day/night cycle. Cage sizes varied (depending on availability) from 1.5m x 2.5m to 6m x 8.5m. Nine cages were constructed for filming the animals' nocturnal behaviour. They had an average size of 3m x 3m. More detailed information on the design of the video cages can be found under 6.2.2 Cage design. All cages contained shelters for the animals consisting of either roofed areas and/or wooden nesting boxes. Shade cloth was provided as sun protection in very exposed areas during the summer months. It was also used as a visual barrier within and between cages. All cages required a ceiling to prevent the animals from climbing out. Cage flooring was either soil or concrete. Both types required different kinds of environmental enrichment to satisfy the continuous need for stimulation of captive animals in order to prevent the development of stereotypic behaviour.

Cages with a concrete floor were supplied with a thick layer of hay bedding to satisfy the animals' digging behaviour. A variety of nesting materials was provided (hay, straw, gum bark, fresh grass preferably with soil covered roots). Wooden logs, gum bark and leaves were placed in varying positions, offering the opportunity to investigate different textures and smells. Soil dishes, containing large 'pot' plants at times, were trialed to encourage digging and ex-

ploration behaviour. Additional food dishes were either hidden or placed in varying positions. The placement of 'treats' (e.g. nuts) into cat treat balls (WLPET, China), encouraging animals to work for their food, has been very successful as part of this thesis. Using the same principle, peanuts were left in the shell, stimulating investigation and manipulation skills as well as providing a food reward.

Introducing native plants wherever possible enriched cages with a soil floor by providing additional shelter, food sources and investigation opportunities for the animals. Depending on the conditions of each cage, native trees such as *Acacia*, *Allocasuarina* and *Eucalyptus* species as well as grasses, rushes and sedges were planted (Appendix A.1 Native plants). A sprinkler system was installed on the cage ceiling, ensuring adequate soil moisture for plant growth. Disturbance of the soil in varying locations as well as the introduction of mushroom compost were used for enhancing digging and investigation behaviour. Varying materials for exploration as well as methods of food acquisition were continually introduced as described above for concrete floor cages.

2.2.6 Housing

Potoroids are generally described as solitary animals with the exception of associations during mating and between mother and young (Seebeck & Rose 1989). When held as a pair in captivity, bettong females have been described tolerating males only at oestrus while displaying aggressive behaviour towards them at other times (Virtue 1987). Captive potoroos on the other hand are reported to rarely fight (Dempster 1965), with pairs resting together and maintaining body contact (Ullmann & Brown 1983).

The above behaviour was observed in both species while in care at the University's captive colony. Breeding bettongs were housed in pairs or in a harem-style with one male having access to several females, provided sufficient cage space was available. Subadult sons were usually tolerated by the adult male, but were separated before reaching sexual maturity to prevent injury and possible inbreeding. One nest box per animal was provided since only mothers

shared the nest with their offspring. Bettong females, participating in the video section of this study, were housed individually with their young to shift the focus to mother-young interactions.

All potoroos within the captive colony displayed a high degree of social behaviour and were therefore housed in harem-style groups. Being held as a group appeared to contribute to stress reduction and was therefore applied to all potoroos whenever possible regardless of their purpose within the project.

2.2.7 Nutrition

Prior to commencement of this project, the bettong diet consisted of dog food pellets, apples and water, occasionally substituted with bread (Animal House management). The well-being of the potoroos could not be maintained with this diet due to rejection of the provided food and subsequent weight loss. Communication with other institutions and testing new foods led to the development of the following diet used for both species. This contained a large amount of fresh vegetables (particularly root vegetables), finely sliced mushrooms in addition to a small amount of fruit and oats as well as mealworms, boiled egg, nuts and seeds. Lucerne hay was provided, especially if there was no fresh grass available. A sprinkler system supplied the appropriate degree of soil moisture for the establishment of wild mushrooms and insects, representing a 'natural' food source for the study animals. A salt-lick was positioned in every cage, although it seemed to be used only by few individuals on occasions.

Vegetables and fruit were chopped into approximately 1cm cubes, enabling young animals to manipulate their food and carry it away if necessary. Fresh water was freely accessible for the animals at all times. Food was provided three times a week when all bowls were cleaned thoroughly. Water bowls in cages with large pouch young or small young-at-foot contained a large rock to avoid accidental drowning of offspring.

2.2.8 Capture and handling

The repeated capture and handling of study animals in captivity is unavoidable for maintaining a healthy population. An experienced quick and confident, though gentle approach is usually applied to prevent distress and injury for handler and animal (Walraven 1994).

After locating the nesting site of the animal to be captured, a hand-net was placed directly over the nest's entrance. Gentle movement of nesting material at the other end of the nest usually induced the animal to escape in the opposite direction ending up in the net. The animal was quickly removed from the net to avoid injury, being held by the tail. It was important to hold the middle of the tail for the safety of both investigator and animal. If held towards the end of the tail the degree of control over the animal decreased making it vulnerable to injury or being dropped by accident, whereas being held towards the base of the tail increased the chances of getting bitten due to the animal's ability to curl upwards. Since these animals are capable of delivering powerful kicks with their hind legs, it is advisable to keep them at a safe distance when handling.

Capture provided a good opportunity for a general examination, for example obvious injuries, diseases or parasites. In case of a female, a gentle stroke across the pouch area indicated if a pouch young was present and, if so, whether further investigation was needed. In case of intense struggling, the captured animal was placed back on the ground for the routine examination, ensuring a firm grip on its tail. If an animal escaped the initial capture attempt, it was given time to hide in another nesting site before initialising further attempts. Much care was taken not to chase the animal over prolonged periods of time. If an animal could not be caught in a relatively stress free manner, it was excluded from data collection, being rescheduled for another day.

For restraint, measurement collection and/or short distance transport purposes, adults were placed in hessian bags, younger animals in pillowcases. An identification number was attached to each bag to ensure that the right young was returned to the appropriate mother in case it had not yet received a microchip.

Both types of bags were turned inside out due to the animals' habit of manipulating the stitched edge. Ingested fibres can cause stomach blockages (Austin 1997). Loose threads can wind around body parts cutting off blood as well as air circulation (Woods 1999, this study). Hessian bags were washed regularly to remove contaminations as well as reduce dust occurrence when handling the animal within the bag.

2.2.9 Restraint

2.2.9.1 Physical restraint

When necessary, an animal was quickly restrained within the bag by being led head first into one of the corners, with the bag material tightened around it, preventing it from turning around. The bag was turned upside down while supporting the animal's body. It was placed between the investigators legs, kneeling on a foam mat. The animal would then be positioned lying on its back with feet and tail towards the opening of the bag. The operator's leg pressure was adjusted according to the individual level of restraint needed. The bag could then safely be opened, holding the animal's feet and tail with one hand while the other was free for obtaining measurements, removing pouch young or administering injections. Younger animals required less restraint and were only kept in the bag to minimise stress and prevent them from injury while being in an unknown laboratory environment.

2.2.9.2 Chemical restraint (sedation and anaesthesia)

Sedation was trialed for the acquisition of milk samples since physical restraint alone led to stress related problems in both milk production (reduction of obtainable amount) and re-attachment of young. The initially chosen form of sedation (*Pamlin injection*, active constituent: Diazepam 5mg.ml^{-1}, dose rate for bettongs and potoroos: 2ml.5kg^{-1}) was not effective on potoroos. The side effect of muscle relaxation had a negative effect on the control of the pouch muscles after the pouch young was returned. Further difficulties were experienced with non-retention of young, which could not be prevented by taping of the pouch.

Following veterinary advice, it was decided to implement the use of isoflurane in oxygen (IsoFlo™ Inhalation anaesthetic; Ohmeda (Isotec 3), England) for stress reduction when collecting milk samples or handling small pouch young. The gas was administered through a black rubber mask held over the animals face (Appendix A.2 Anaesthesia settings). Isoflurane was turned off at the end of each procedure and the animals were given pure oxygen to breathe via the same mask while regaining consciousness and muscle control within minutes. In contrary to the effects of diazepam (above), they did not require prolonged periods of supervision after release back into their cages. The use of isoflurane in this project facilitated the collection of sufficient milk samples and improved effective re-attachment of the young.

2.2.10 Injuries and diseases

Little information is available on disease treatment and appropriate dose rates for bettongs and potoroos. Although strict hygiene, routine and stress minimisation were main husbandry principles, the following diseases were encountered during this project. Dr R. Woods, Dr B. Gartrell and Dr E. Wronski provided veterinary treatment.

Fight wounds in male and female bettongs were common injuries. Although sometimes quite severe, these wounds usually did not require veterinary treatment (unless infected), but the particular animal was separated for recovery purposes. Two cases of leg fractures were encountered, entailing surgery. One animal died under anaesthetic while the other recovered well after insertion of metal pins, eventually resulting in the stiffening of the ankle.

Cases of diarrhoea were treated with *Protexin Professional* (concentrated multi-strain probiotic). The control of tick infestations was achieved by usage of *Advantage* (active constituent: 100g.l^{-1} Imidacloprid, dose rate for bettongs and potoroos: 0.1ml.kg^{-1}, applied to the back of the neck). Mite infestations spreading through the whole colony were alleviated by applying *Cydectin* pour-on for cattle and deer* (active constituent: 5g.l^{-1} moxidectin, dose rate for bettongs and potoroos: 0.1ml.kg^{-1}, applied to the back of the neck). *Ilium Fung-*

fite Antifungal Cream was administered to exposed skin areas affected by ringworm (active constituent: 20mg.g^{-1} miconazole nitrate). Cases of coccidiosis were managed with *Baycox Coccidiocide Solution* (25mg.kg^{-1}, dose rate for bettongs and potoroos: 1ml.kg^{-1}), which was mixed with blackcurrant syrup to improve palatableness without drastically increasing the amount to be orally administered.

The rare cases of pneumonia and toxoplasmosis (Appendix A.3 Toxoplasmosis) as well as myopathy (during rehabilitation) had rapid lethal consequences, therefore treatment options were considered not viable. A potential case of 'Lumpy jaw' in a bettong was reported while in rehabilitation, which led to severe abscess formation. Treatment consisted of a prolonged course of antibiotics (*Baytril*, dose rate for bettong: 0.1ml.kg^{-1}) and subsequent tooth extraction. Another antibiotic used for bettongs was *Clavulox Injectable Suspension* (Pfitzer Animal Health, Amoxycillin140mg.ml^{-1} and Clavulanic Acid 35mg.ml^{-1}, dose rate: 1ml.20kg^{-1}) for the treatment of infections occurring after surgery or on open wounds like damaged toenails. When necessary, blood/faecal analysis and autopsies were performed at the Animal Health Laboratories (Department of Primary Industries, Water and Environment [DPIWE], Mt.Pleasant).

2.2.11 Record keeping

Records were kept of data gathered for all individual animals including personal identification (ID), cage number, body measurements, number of obtained milk samples, development, reproductive status, ID of produced offspring, illnesses, medications and fate as well as weather conditions and other potentially influential factors. Since this data collection only included a selection of animals per day, a walk through the colony was performed each night to ensure the well-being of all individuals. Animals could be observed without handling. The qualitative information gathered from their display of individual and social behaviour (rather than their physical appearance alone) enabled an early detection of 'problem animals' (e.g. problems with feeding or social integration).

These records were considered to be essential for the maintenance of a healthy captive population and the establishment of a successful breeding program. The latter formed the basis for this project of pouch young transfers, but also ensured the reduction of animals sourced from wild populations for research purposes (Appendix A.4 Data collection sheet).

2.2.12 Pests and other unwanted species

The main vermin species were mice (*Mus musculus*) and rats (*Rattus rattus*) in large numbers occassionally as well as sparrows (*Passer domesticus*), sharing the study animals' food supplies and contaminating their environment. Liquid poison (*Bromakil*, Hoechst Schering AgrEvo, active constituent: 0.5g.l^{-1} Bromadiolone, dose rate: 100ml.900ml^{-1} water) was used for vermin control. Poison pellets were laid in other sections of the enclosure and pellet parts, dropped by rats, as well as food stashes, created by mice, containing poison could be found in various locations. The risk of study animals coming into contact with poison was therefore minimised by using a liquid rather than pellets.

Some cages were more affected by vermin than others, due to soil flooring and larger size gauge wire, enabling vermin to either dig their way into the cage or simply walk through the wire. Rats demonstrated dominance over the study animals, reaching the size of a small potoroo. None of the study animals died, but the vermin presence clearly had an impact on their displayed behaviour (6.3.2.8 Social behaviour including others). On rare occasions Tasmanian Tiger Snakes (*Notechis ater humphreysi*) were spotted in the enclosure, causing no harm to bettongs or potoroos.

2.2.13 Data management and statistical analysis

The software package Microsoft® Excel 2000 was used during the data collection process for milk results, growth measurements, development of young and maintenance data for captive colony management purposes. Behavioural data was processed with the software "The Observer" (version 4.1.126, Noldus Information Technology, The Netherlands). All gathered information was com-

bined in a database using Microsoft® Access 2000 and subsequently analysed using the statistic software SPSS 12.0 for Windows. Statistical manuals (Lamprecht 1992, Pallant 2001) were used for the data analysis. Transfer categories (cross-foster, foster and original young for both species) as well as transfer age differences per category were tested for significant differences by performing a One-Way-ANOVA with subsequent *post-hoc* comparison (Least Significant Difference t-Test, 0.05 level of probability) for each individual pouch age (milk), age of young (growth and development, up to 52 weeks) and week after pouch vacation (behaviour). Analyses were performed under the guidance of Dr D. Ratkowsky (University statistician).

Chapter 3: Pouch Young Transfers

3.1 Introduction

The application of pouch young transfer in marsupials has indicated several advantages as well as limitations, which have to be considered to ensure the survival and well-being of the transfer young while maximising the female's rate of production of young. Johnson (1981) formulated four criteria for a careful selection of intended transfer young and mothers, which need to be addressed prior to the transfer procedure. Firstly, calm transfer mothers were chosen. Secondly, original parent of the transfer young and transfer mother were of similar size to prevent the young from outgrowing the pouch before pouch vacation. Thirdly, the transfer age difference (TAD) between the young of the transfer mother and the intended transfer young was kept to a minimum to increase the survival rate of the latter. This point was particularly stressed, given the high death rate for young with a TAD of more than 20 days as reported by Clark (1968): this was related to the changing milk composition and its possible unsuitability for the particular young. Fourthly, pouch young transfer in late pouch life was avoided since preliminary observations indicated a rejection of older young.

Once the decision is made for the appropriate transfer young and mother, the next critical step in pouch young transfers is the detachment of the young from its original teat and the subsequent attachment onto the teat of its transfer mother. Merchant and Sharman (1966) found that the young marsupial's mouth was modified to accommodate the bulbous swelling on the end of the teat between hard palate and tongue. This resulted in a firm attachment of the young to the teat aided by the fusing of the lips. The size of the teat increases with the growing young in the pouch, which represents a limitation for pouch young transfers (see Fig.3.2), since teat size needs to be comparable with the mouth opening of the young. Clark (1968) assumed that the death of the youngest transfer young in her study might have been due to a teat that was too large for the young to attach to.

Once the young begins to suckle from the teat of the transfer mother, it is unknown if pouch young can detect the difference in milk composition. Although not a prominent feature in the newborn marsupial, taste buds were found in a small selection of animals for example *Isoodon macrourus* (Peramelidae) and *Macropus eugenii* (Macropodidae) (Hughes & Hall 1988). Morphological evidence suggests that the marsupial newborn uses the senses of gravity, odour and touch when travelling from the birth canal to the pouch opening (Gemmell & Rose 1989). The authors noted that defined olfactory bulbs are present in both newborn Tasmanian bettongs and Long-nosed potoroos. Pouch gland secretion might play an important role in humidifying the marsupium and assisting the newborn in locating the pouch after birth as well as providing identification odours for the young (Salamon 1996). The taste of the milk as well as the pouch odour of the transfer mother might therefore have a considerable impact on the success rate of re-attachment in transfer young.

Pouch young transfers can be used for accelerating the female's reproductive rate or assist in the management of species, which do not successfully rear their offspring in captivity. The management of threatened species requires ethical considerations in terms of either leaving the individuals of interest in their fragile wild environment or bringing them into captivity for controlled attempts to increase the population size while possibly jeopardising their ability to adapt to changes in their environment later on (Primack 1995). A compromise can be achieved by only retrieving the pouch young without the wild donor mother undergoing the stresses of captivity (e.g. transport, handling, movement restrictions, artificial environment and diet etc.). Johnson (1981) was successful in transporting young to the captive colony with travelling times varying between 4 and 30 hours. Taggart *et al.* (2002) points out the potential of combining wild pouch young isolation (threatened species) and subsequent transfer in captivity (to a common species) for increasing animal numbers and improving genetic diversity in captive breeding programs while accelerating the breeding in the original females in the wild by activating the dormant blastocyst.

The aim of this study was to investigate the applicability of pouch young transfer to the Tasmania bettong and Long-nosed potoroo with particular interest in the possible impact of transfer age difference on survival rate in young and subsequent reproductive success once mature.

3.2 Methods

3.2.1 Animal selection

Pouch young transfers usually require the removal (euthanasia) of the young from the common species to accommodate the transfer young of the target species. None of the young were euthanased as part of this study, since the transfer of both young provided data for both species simultaneously. Therefore no information could be gained on a possible increase of the mother's reproductive rate.

Pouch young transfers were initially performed within each species (bettong-bettongs, potoroo-potoroo), which is referred to as 'intra-species transfer or fostering'. Once transferred young were accepted and reared by their foster mothers, pouch young transfers were carried out across the species level (bettong-potoroo, potoroo-bettong), which is referred to as 'inter-species transfer or cross-fostering'.

Unknown ages of pouch young were estimated from growth data collected by Rose (1984) for the Tasmanian bettong and Bryant (1982) for the Long-nosed potoroo in addition to data gathered for this study. The transfer of young was performed at different stages of pouch life. In this project pouch life was divided into three stages: early (pouch age 1 to 4 weeks), middle (pouch age 5 to 9 weeks) and late pouch life (pouch age 10 to 15 weeks for bettongs or to 17 weeks for potoroos). The intended transfer young either had the same age or differed in age up to three weeks (Fig.3.1).

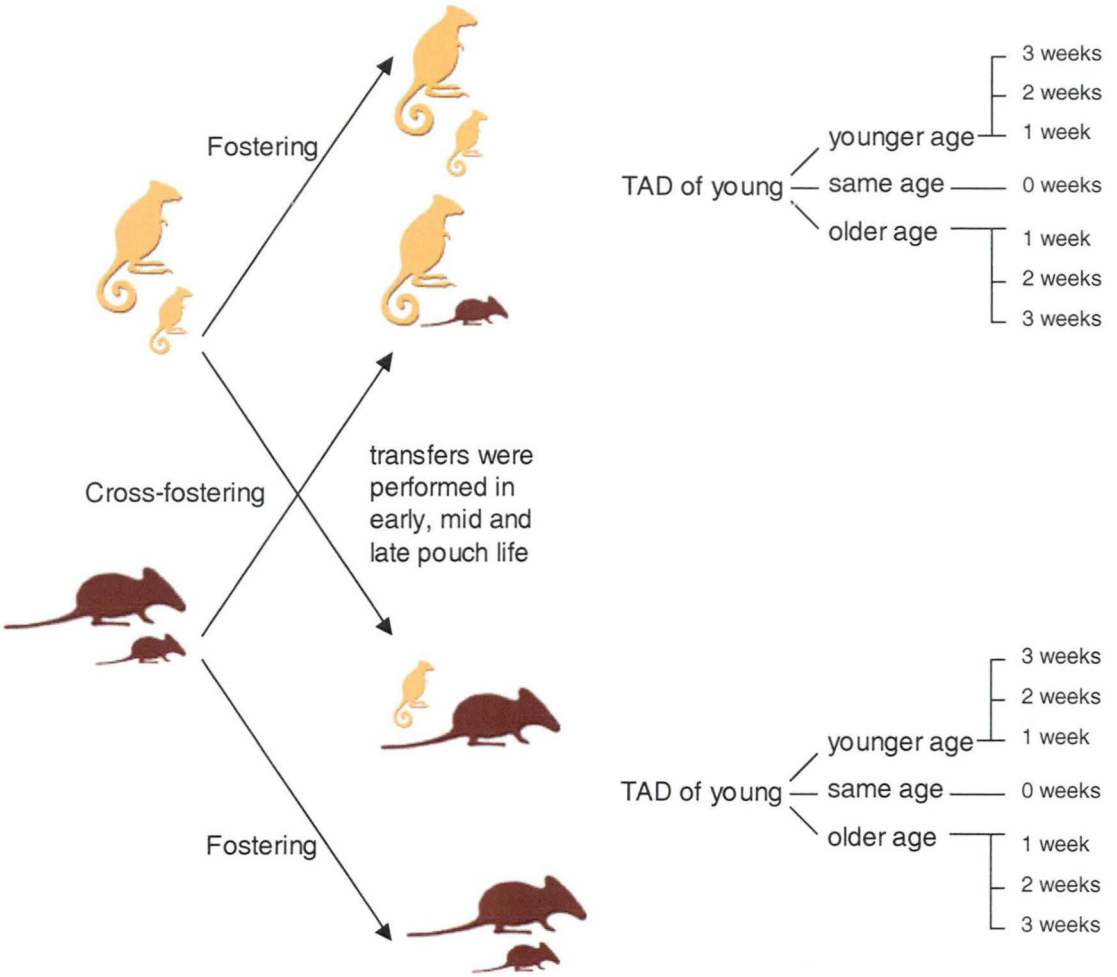

Fig.3.1: Variety of options for intra-species (Fostering) and inter-species (Cross-fostering) transfers given as age of young at time of transfer and transfer age difference (TAD) between transfer young in weeks (bettong symbols: yellow, potoroo symbols: brown).

The age difference for transfers in early pouch life did not exceed one week due to physical limitations in size of teat and mouth opening of the young involved (Fig.3.2).

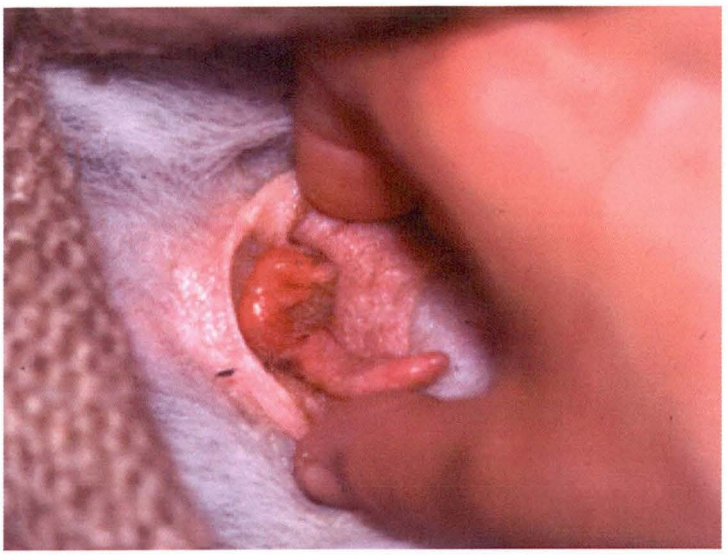

Fig.3.2: The pouch young is firmly attached to its teat. The bigger teat on the right is used by the young-at-foot (YAF) drinking from the outside of the pouch. It would not fit into the mouth of the pouch young.

3.2.2 Transfer technique

Mothers were anaesthetised using Isoflurane in oxygen (2.2.9.2 Chemical restraint) to reduce the risk of injury to the young during the transfer procedure. Young are very fragile, especially in the first weeks following birth. The level of support and/or restraint required for transfer depended to a great extent on their age.

The head of the young was placed between thumb and index finger, while the other fingertips supported its body by gently holding it against the thumb. Index finger and thumb of the other hand held the teat, which the young was firmly attached to. Both hands were subsequently rotated in opposite directions, gently twisting the head of the young as well as the teat while slowly pulling them apart. Great care had to be taken to avoid pulling the young off the teat too quickly or applying too much pressure when handling the young. Failure in acting accordingly could have damaging or even lethal effects (Gates, pers. comm.) on the well-being of the young.

Older young were safely pulled out of the pouch first before being taken off the teat due to their size. This was achieved by positioning the young in the pouch to prevent twisting of the body when being pulled through the pouch opening, while the mother was restrained (see 2.2.9.1 Physical restraint). Both legs and tail of the young were held together and gently pulled out of the pouch at an angle comfortable for the young. Once the young was taken safely off the teat, body measurements were quickly obtained (5.2.1 Body measurements). It was also noted from which teat the young was taken to ensure that the transfer young would be aided in re-attaching to the appropriate teat.

The interior of the mother's pouch was wiped with a cotton swab. The young was wrapped up in the scented swab and held onto the skin of the investigator while the transfer mother was anaesthetised for removing her young. The second young was treated in exactly the same way as the first one until both young were wrapped up in their individual cotton swabs (Fig.3.3a). They were kept warm via the investigator's skin due to failure in maintaining their body heat when placed in a humidicrib on their own.

The original cotton swab was placed in the pouch of the transfer mother to introduce some of the scent from the original mother. After removing the swab the young was aided in re-attaching to its new teat. Both young and teat were held in the same way as described in the removal process above. The young was first placed into the pouch to provide it with security and body heat. Only the head of the young as well as the appropriate teat were kept in the pouch opening to restrain the young from movements, which would interfere with the procedure. Once the new teat was placed onto the mouth of the young, a plastic sheath (Fig.3.3b) was used to gently push the teat into the small mouth opening.

As soon as an acceptable attachment was achieved, the pouch opening was closed gently. The mother was breathing pure oxygen, while her recovery was supervised. After regaining complete consciousness and muscle control the female was released into her cage and left undisturbed. The well-being of the

young was checked one week later. It was not removed from the teat for the purpose of milk sample collection until it reached an age of seven weeks. When collecting milk samples from the mother during the following weeks of pouch life, young were sometimes found in the pouch detached from the teat. Although this was indicating its ability to re-attach without assistance, the young was always aided back onto the appropriate teat after milking. Additional pouch swabs were taken for a preliminary analysis of pouch odour, which provided inconclusive results and subsequently this had to be abandoned due to time constraints.

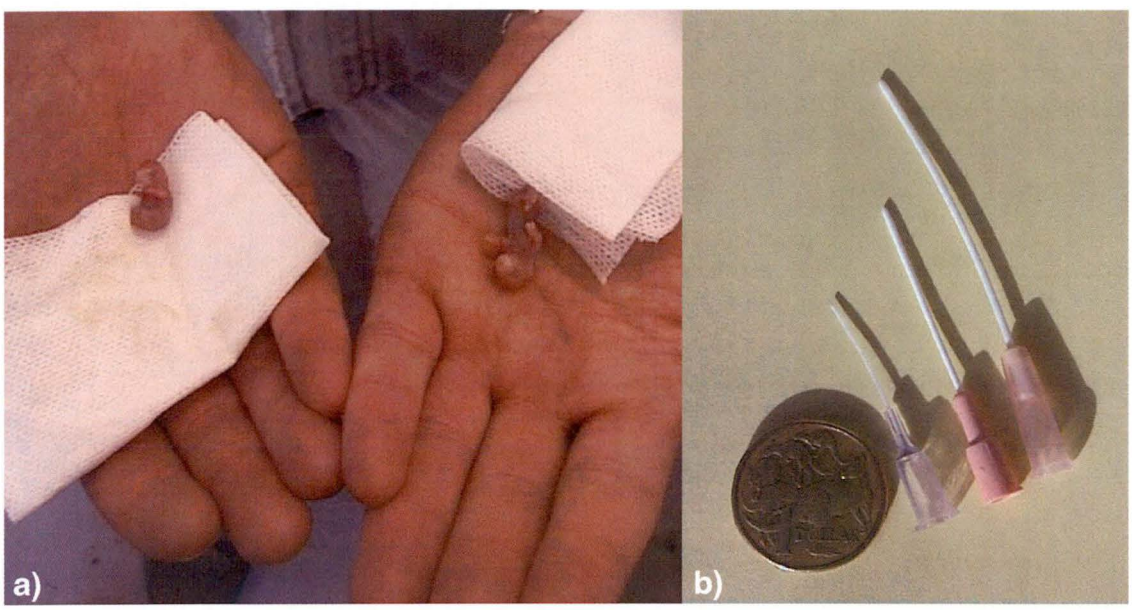

Fig.3.3: a) Pouch young with their scented cotton swabs after removal from the teat (bettong left, one week old, potoroo right, two weeks old), b) plastic sheath selection (for different size mouth openings).

3.3 Results

A total of 32 pouch young transfers have been performed. They divided into 18 intra-species transfers (bettong-bettong: 16, potoroo-potoroo: 2) and 14 inter-species transfers (bettong-potoroo: 7, potoroos-bettong: 7). All surviving young produced their own offspring either with a different transfer animal or another member of the captive colony.

3.3.1 First interactions of transferees

At birth the forearms of the young are very well developed to enable them to climb from the birth canal to the pouch opening unaided. Leaving the forearms of the young unrestricted in the re-attachment process usually resulted in the young pulling the teat out of its own mouth. The plastic sheath was only used to insert the tip of the teat into the mouth opening of the young, since most young achieved proper re-attachment via their own sucking action.

There were several incidences where the young actively detached itself from the transfer teat during the transfer procedure. Since this might have been due to a change in milk composition, a drop of the original milk was placed on the teat to encourage the transfer young in accepting the new teat. This was not an option for the transfers in early pouch life, since mothers produced only small volumes of milk. On several occasions no firm attachment of the young to the teat of the transfer mother could be achieved.

In most cases the first interactions between young and transfer mother occurred during the recovery process from the anaesthetic. Initially females inspected and then licked the pouch opening, subsequently smelling and licking the transferred young. No signs of aggressive behaviour could be observed at this stage in any of the 32 transfers.

3.3.2 Mortality

Deaths were recorded for both transfer categories in each species (bettong: 4 out of 16 foster young, 5 out of 7 cross-foster young; potoroo: 1 out of 2 foster young, 1 out of 7 cross-foster young) (Table 3.1). Pouch young losses were recorded for young transferred in early (bettong: 4, potoroo: 1) and mid pouch life (bettong: 5, potoroo: 1). The transfer age difference did not appear to be closely related to the mortality rate. Two same aged potoroo young died and bettong losses were recorded in nearly every TAD category (-3 weeks: 3 young, -2 weeks: 1 young, -1 week: 1 young, 0 weeks: 2 young, 1 week: 1 young, 2 weeks: 1 young).

Death occurred in four bettong young in the first week following the transfer procedure; three further young were lost in the following two to four weeks. One bettong and two potoroo young died between week 5 and 10 following the procedure. The oldest bettong young was lost 23 weeks after being transferred. Young that died more than six weeks after the procedure, were transferred in early pouch life (age at transfer: 1 week, TAD: 0 and –1 week). Autopsies were performed to rule out diseases, but were inconclusive. Dead young, however, were not always found (in some cases due to cannibalism). On two occasions rejected young were found alive on the cage floor. One of them was hand-reared successfully.

Table 3.1: Species, transfer type (F=foster, CF=cross-foster), sex and fate for each transfer animal accompanied by the age of the young at time of transfer, the transfer age difference (TAD, negative value = younger, positive value = older) between the transfer young as well as the age of the young at time of death in weeks. # = death was not due to pouch young transfers. Re-use = animal remained in the captive colony for future research.

animals' ID	species	type	sex	transfer age	TAD	fate	age at death
F-M BetA	bettong	F	male	6	-3	dead	9
40610B3F14	bettong	F	female	9	-2	released	
F-M Bet11	bettong	F	male	2	-2	dead#	
F-M BetE	bettong	F	male	1	-1	dead#	
'Halfblind'	bettong	F	female	9	-1	released	
4061154D74	bettong	F	male	6	-1	released	
40603F5036	bettong	F	female	7	0	released	
40603B0427	bettong	F	female	7	0	released	
F-F Bet8	bettong	F	female	5	0	released	
F-F BetC	bettong	F	female	5	0	dead	6
40607F5B06	bettong	F	female	10	1	released	
4060746A7C	bettong	F	male	7	1	released	
F-M BetB	bettong	F	male	2	1	dead	3
4060551F42	bettong	F	female	11	2	released	
F-M BetD	bettong	F	male	4	2	dead	8
4061214D03	bettong	F	male	9	3	released	
CF-M BetA	bettong	CF	male	9	-3	dead	11
CF-M BetD	bettong	CF	male	9	-3	dead	10
422E325E60	bettong	CF	male	2	-2	re-use	
CF-F BetB	bettong	CF	female	8	-2	dead	9
CF-M Bet1	bettong	CF	male	4	-1	released	
CF-M Bet3	bettong	CF	male	1	-1	dead	24
CF-F BetC	bettong	CF	female	1	0	dead	10
F-F Pot1	potoroo	F	female	9	0	released	
F-F PotA	potoroo	F	female	9	0	dead	15
CF-M PotA	potoroo	CF	male	1	0	dead	9
CF-M Pot2	potoroo	CF	male	5	1	released	
CF-M Pot3	potoroo	CF	male	2	1	released	
CF-F Pot1	potoroo	CF	female	10	2	released	
CF-F Pot2	potoroo	CF	female	4	2	released	
CF-F Pot3	potoroo	CF	female	12	3	released	
CF-M Pot1	potoroo	CF	male	12	3	released	

3.3.2 Foster transfers

Breeding difficulties were experienced in the early part of this study, which might have been due to spatial housing limitations. Continuous breeding by re-activating the dormant blastocyst was a rare occurrence within the colony and was not observed terminating the pouch life of any transfer young. All transfer young were allowed to remain in the pouch for the full term of pouch life (appro-

priate for the transfer mother), which was extended up to 2 weeks (Table 3.2). Three of the younger foster bettongs had to leave the pouch at pouch age 15 weeks when pouch vacation usually occurs. This was of no relevance for the two males (TAD: -1 week), since their growth and development was considered appropriate to advanced for their age. The younger female, however, was lacking in growth and development compared to the pouch age (TAD: -2 weeks). She was rejected by the foster mother after pouch vacation and subsequently hand-reared. All surviving young that reached sexual maturity produced their own offspring.

No consistent relationship could be established between the above presented age parameters (e.g. transfer age, TAD, age of young at time of PEP) and the age of mature transfer young that became reproductively active. Three foster bettong young produced their first own offspring comparatively early in life (age: 24 to 27 weeks) compared to other bettong young.

Table 3.2: Species, sex, age at time of transfer and transfer age difference (TAD) for each individual foster young accompanied by pouch age and age of young at time of permanent emergence from the pouch (PEP) as well as age at time of birth of first own young in weeks.

animals' ID	species	sex	transfer age	TAD	pouch age/PEP	age/PEP	age/1.PY
40610B3F14	bettong	female	9	-2	15	13	25
F-M Bet11	bettong	male	2	-2	16	14	NA
F-M BetE	bettong	male	1	-1	15	14	NA
'Halfblind'	bettong	female	9	-1	16	15	52
4061154D74	bettong	male	6	-1	15	14	49
40603F5036	bettong	female	7	0	16	16	38
40603B0427	bettong	female	7	0	16	16	27
F-F Bet8	bettong	female	5	0	15	15	24
40607F5B06	bettong	female	10	1	15	16	35
4060746A7C	bettong	male	7	1	16	17	64
4060551F42	bettong	female	11	2	15	17	37
4061214D03	bettong	male	9	3	15	18	52
F-F Pot1	potoroo	female	9	0	18	18	70

Several primiparous mothers lost their first (and at times second) young, before rearing offspring successfully (Table 3.3). No information on the reproductive rate in transfer young was gained, since animals did not have comparable

amounts of time available for reproduction or ongoing access to potential mating partners due to logistic constraints. Foster bettong young produced a total of 27 young, of which 10 were lost. The ratio of reared offspring was male biased (6 females to 11 males). The only surviving foster potoroo young gave birth to one female and one male young and reared both successfully.

Table 3.3: Species, sex, age at time of transfer and transfer age difference (TAD) in weeks for every individual foster young accompanied by information on their own offspring given as number of pouch young produced ('reared' refers to maternal care and implies the successful completion of pouch life and/or achievement of independence, f=female young, m=male young) and survival. * Indicates that male produced additional young with other foster female; the young is listed with the mother.

animals' ID	species	sex	transfer age	TAD	nr. of PY	reared (f)	reared (m)	lost
40610B3F14	bettong	female	9	-2	6		4	2
'Halfblind'	bettong	female	9	-1	2	2		
4061154D74	bettong	male	6	-1	*1	1		
40603F5036	bettong	female	7	0	2		2	
40603B0427	bettong	female	7	0	4		1	3
F-F Bet8	bettong	female	5	0	2		1	1
40607F5B06	bettong	female	10	1	2		2	
4060746A7C	bettong	male	7	1	*3	1	1	1
4060551F42	bettong	female	11	2	5	2		3
4061214D03	bettong	male	9	3	*			
F-F Pot1	potoroo	female	9	0	2	1	1	

3.3.4 Cross-foster transfers

All cross-foster young remained in the pouch for the full term of pouch life appropriate for the transfer mother's species, which was extended up to two weeks (Table 3.4). Two transfer mothers of cross-foster potoroos prevented the return of their young into the pouch early at the appropriate pouch vacation time for the transfer mother, which had no negative implications for the young given their advanced state of growth and development.

A total of three cross-foster bettongs survived pouch life, but only two animals reached sexual maturity. All cross-foster potoroo produced their own offspring at a comparatively earlier age than the cross-foster bettongs or the foster potoroo.

Table 3.4: Species, sex, age at time of transfer and transfer age difference (TAD) for each individual cross-foster young accompanied by pouch age and age of young at time of permanent emergence from the pouch (PEP) as well as age at time of birth of first produced young in weeks.

animals' ID	species	sex	transfer age	TAD	pouch age/PEP	age/PEP	age/1.PY
422E325E60	bettong	male	2	-2	19	17	65
CF-M Bet1	bettong	male	4	-1	18	17	68
CF-M Bet3	bettong	male	1	-1	18	17	NA
CF-M Pot2	potoroo	male	5	1	15	16	53
CF-M Pot3	potoroo	male	2	1	15	16	51
CF-F Pot1	potoroo	female	10	2	17	19	46
CF-F Pot2	potoroo	female	4	2	15	17	48
CF-F Pot3	potoroo	female	12	3	16	19	60
CF-M Pot1	potoroo	male	12	3	15	18	49

The cross-foster bettong males produced one young each (one female, one male), which were successfully reared by their mothers. Cross-foster potoroos produced a total of nine young with an almost equal sex ratio of three females and four males being reared successfully. Only two deaths were recorded for young of cross-foster potoroo females (Table 3.5). As with the foster young, no information on reproductive rates could be obtained due to logistic constraints.

Table 3.5: Species, sex, age at time of transfer and transfer age difference (TAD) in weeks for every individual cross-foster young accompanied by information on their own offspring given as number of pouch young produced ('reared' refers to maternal care and implies the successful completion of pouch life and/or achievement of independence, f=female young, m=male young) and survival. * Indicates that male produced additional young with other cross-foster female, the young is listed with the mother.

animals' ID	species	sex	transfer age	TAD	nr. of PY	reared (f)	reared (m)	lost
422E325E60	bettong	CF	male	2	1	1		
CF-M Bet1	bettong	CF	male	4	1		1	
CF-M Pot2	potoroo	CF	male	5	1	1		
CF-M Pot3	potoroo	CF	male	2	*			
CF-F Pot1	potoroo	CF	female	10	4	1	2	1
CF-F Pot2	potoroo	CF	female	4	3	1	1	1
CF-F Pot3	potoroo	CF	female	12	1		1	
CF-M Pot1	potoroo	CF	male	12	*			

3.3.5 Reunion with original species

All cross-foster young were separated from their mothers at the end of the weaning process and reunited with their original species. This had different implications for the two species of young, since they encountered altered social structures compared to the mother-young bond. All adult potoroos showed a high degree of sociability in captivity including body contact, allo-grooming and sharing of the sleeping nest (chapter 6: Behaviour, Appendix A.9 Sociability). Adult bettongs on the contrary displayed higher levels of aggressive behaviour towards each other when in close proximity with the exception of mother-young dyads. Cross-foster potoroos appeared to have an advantage in being transferred from a social mother-young dyad into a social potoroo group. Introducing two cross-foster potoroo young of similar age and opposite sex into the group together appeared to facilitate the familiarisation process with the new surroundings and foreign animals. Both young reached sexual maturity and produced several offspring together.

Although not quantified, the separation from the mother appeared to induce the seeking of body contact with other group members for comfort in the cross-foster young. This had detrimental effects on the two cross-foster bettongs males, since they changed from a social group to a more solitary life style. They became the target for agonistic behaviour and suffered a series of injuries when encountering another female. The displayed aggression occurred regardless of the behaviour's nature exhibited by the cross-foster bettong males (e.g. individual behaviour, mating behaviour etc.). They were subsequently paired with young inexperienced females, which prevented further behavioural problems.

3.4 Discussion

No rejection of young was observed at time of transfer. Russell (1982) stated that there is little evidence for individual recognition of marsupial young before they leave the pouch. In a later publication Russell (1989) referred to unpublished observations of females accepting transferred young of the same or different species as well as accepting a second young in their pouch or ex-

changing young. Although she could not determine if the underlying cause for such behaviour was lack of recognition or an acceptance, regardless of recognising a young as strange, she assumed that the young would quickly acquire the "right smell" once in the pouch.

However, previous pouch young transfer studies have shown mortality rates in combination with different age categories. Clark (1968) lost 4 out of 5 transferred young with a TAD greater than 20 days. Johnson (1981) lost only 2 young (TAD less than 1 week) out of 14 transferred animals, but reported no mortality for animals with larger TADs (maximum of 42 days). Attachment failure and interference from males were given as possible causes for the loss of young. Smith (1989) reported one death (TAD: 19 days younger) in ten transferred young. In a later transfer experiment she (Smith 1998) lost 2 out of 11 transferred animals (TAD: 0 and 1 day respectively), one appeared unhealthy at time of transfer and died four weeks after transfer, the other one at 80 days old.

Although no information is available for the existence of taste buds in the newborn or pouch glands for Tasmanian bettongs or Long-nosed potoroos, the observations made during the re-attachment process of the transferred young suggest an active participation of the transferred young in accepting or rejecting the new teat, which could be based on chemical clues. This might have implications for the mortality seen in the first week following the transfer procedure, usually commented on as attachment failure (e.g. in Merchant & Sharman 1966, Johnson 1981), which could in fact be attachment rejection.

The loss of young several weeks after transfer could be related to the young's inability to make use of the milk's nutrients provided by the transfer mother, which do not necessarily reflect the needs of the suckling young at the time depending on the TAD (especially relevant for three weeks younger transferees). However, the high survival rate in the present study of young transferred in late pouch life could be due to their more advanced development, which might be accompanied by improved capabilities in digesting milk at a different lactational stage or in better dealing with stress.

The initial breeding diffculties in this study, probably due to overcrowded housing conditions, and occurences of mothers eating their young have been mentioned in previous literature. Tyndale-Biscoe (1968) reported a pouch young mortality rate in captive *Bettongia lesueur* of nearly 50% before young reached an age of 10 days and nearly 70% respectively before they were 20 days old. A behavioural, stress related response of the mother (destroying or eating young) was considered the most likely cause due to their inability to establish territories in confined cage space. Maynes (1973) noted the connection between overcrowded enclosures and low fertility in captive animals and suggested as possible mediators nutritional stress (affecting intra-uterine development), active removal of pouch young by the mother and loss of young while in transit between birth canal and pouch opening.

The length of pouch life varied, but was in most cases extended to suit the needs of the transferred young, as found by Merchant and Sharman (1966), Clark (1968) and Johnson (1981). However, three younger foster bettongs and two cross-foster potoroos had to leave the pouch at the appropriate pouch vacation time for an untransferred young (15 weeks), even though no following young was about to occupy the pouch. Rose (1986) and Smith (1989) found that foster young were excluded from the pouch on the night of parturition of the following young and/or oestrus of the recipient mother, regardless of age and weight of the young. Janssens and Rogers (1989) suggested that the increasing loss of thermal compatability between mother and furred pouch young might play an important role in pouch vacation. This could apply to this study's transferees that showed accelerated growth and development. Rose (1986) referred to the exclusion of a 'full term' pouch young without associated birth of a following young as a "perhaps ... failsafe mechanism".

It was important that matured transferred young not only mated successfully, but also reared their offspring to independence. In a conservation program this would strengthen the colony or provide potential candidates for release into the wild (see also Appendix A.10 Rehabilitation and release). The earliest age for successful reproduction in a transferred young was recorded with 24 weeks for

a foster bettong transferred at the age of five weeks with no TAD compared to the young originally inhabiting the pouch. Rose (1989) stated that Tasmanian bettongs reach maturity within nine months and Long-nosed potoroos within twelve months. Three foster bettong young reached sexual maturity comparatively earlier; however, several female young lost their first and/or second young soon after birth. This failure to rear young successfully might be due to a lack of experience. Russell (1989) suggested that the more important aspects of maternal behaviour are "built-in", but also stated that it is unknown to what extent experience contributes to a female's success in rearing her young.

The sex ratio of offspring produced by mature transferred young was biased towards males for bettong young and about equal for potoroo young. The relevance of these results is not to be overrated due to restricted opportunities to investigate the reproductive rate and is therefore not commented on. Sex ratio variations, however, have been discussed by Johnson and Jarman (1983) in terms of geographical variation, in particular rainfall, and Cockburn (1990) for the theory of sex allocation, local resource competition and the Trivers-Willard hypothesis.

Johnson (1981) used quiet temperament as a selection criterion for potential recipient mothers. The mature transferred potoroos were much calmer than their mothers caught in the wild. They only lost 2 out of 11 young, while their mothers lost all but two young when brought into captivity. The calm nature of the matured transfer animals would have made them better potential transfer mothers, which, however, could not be investigated within the timeframe of this project.

Chapter 4: Milk composition

4.1 Introduction

The composition of milk has been the subject of investigation over the years for various species of eutherian mammals (for example in Linzell 1972 and Jenness & Sloan 1970) as well as monotremes (Griffiths 1965) and marsupials, for example the Phalangeridae (Gross & Bolliger 1959), the Petauridae (Munks *et al.* 1991), the Peramelidae (Merchant & Libke 1988), the Macropodidae (Lemon & Barker 1967, Messer & Mossop 1977, Messer *et al.* 1984) and the Potoroidae (Smolenski & Rose 1988, Crowley *et al.* 1988, Rose *et al.* 2003).

Green and Merchant (1988) pointed out that marsupial milk composition changes dramatically during lactation within a species while remaining relatively uniform between species, contrary to eutherian milk composition, which remains relatively uniform within a species (once full lactation is established), but differs greatly between species. Although the precise relationship between changes in milk composition and development of the marsupial young is still not fully understood, qualitative and quantitative changes in the milk composition give an insight into the nutritional requirements of the developing young (Green & Merchant 1988).

Asynchronous intra-species transfers and inter-species transfers therefore pose the risk of compromising the young's survival by providing the transfer young with unsuitable milk for its age. Merchant and Sharman (1966) argued that retarded growth of younger transfer animals might have been due to unsuitability of milk, while the accelerated growth of older transfer young could have been caused by better usage of the milk or consumption of greater quantities. Young with retarded growth in the transfers performed by Johnson (1981) were successfully supplement fed. The latter author also indicated that disturbance was kept to a minimum.

Stress, especially in the long-term, can have an impairing effect on the immune function and lead to a substantial reduction of milk yields by reducing mammary blood flow and interfering with the action of oxytocin (Akers 2002), which would compromise the health of both mother and young.

The aim of this study was to investigate if intra-species and inter-species pouch young transfer have an effect on the milk composition produced by the transfer mother, with particular consideration of the transfer age difference of young defined in weeks (this part of the study was conducted before Trott *et al.* [2003] published their results).

4.2 Methods

4.2.1 Milk Sample Collection and Storage

Milk samples were collected once per week, or once per fortnight if the animal was highly stressed (e.g. excessive kicking, hyperventilating). Females were caught (2.2.8 Capture and handling) and separated from their young for three hours to allow milk accumulation in the mammary gland. Difficulties can sometimes occur when re-attaching a pouch young younger than five weeks of age back to its teat (Rose pers.comm.). For this reason, pouch young removed from the teat for the purpose of milk sample collection were at least five weeks old. If a young had been transferred at approximately this age, it was not taken off the teat any earlier than two weeks after the transfer occurred. Detailed information on removing young from the teat as well as the age of transferred young can be found in chapter 3 Pouch Young Transfers.

Unfurred young were transferred into a labeled sock or a small cotton bag after being removed from the pouch and then placed in a humidicrib set at a temperature of 37°C. If pouch young failed to maintain their body temperature within the humidicrib (cold to touch or lethargic appearance) or appeared to be very unsettled, they were placed under the shirt of the investigator. The provided body heat and heartbeat calmed the young down very quickly (Appendix A.5 Calming effects). Young-at-foot were able to maintain their own body temperature and could therefore be kept in pillowcases at room temperature.

A whole series of factors contributed to a stressful environment for the animals involved in the milking procedure during the three hour waiting period in the laboratory; disturbance at resting time, exclusion from their accustomed environment, separation of mother from young, being handled and exposure to unfamiliar sounds and smells. A calming effect was achieved by placing all bags holding females or young on cup hooks positioned on the edge of the laboratory bench, enabling them to hang free. This position was possibly soothing for the separated young by simulating the feeling of being in the pouch. For the adult females, it withdrew a hard substrate such as the floor or a wall to use for kicking. It also provided adequate support for recumbent animals recovering from the initially trialed sedation with diazepam to prevent 'disuse muscle atrophy' (Gartrell pers. comm.).

Since milk acquisition from restrained or sedated animals proved to be of limited success (2.2.9.2 Chemical restraint), isoflurane in oxygen (IsoFlo^{TM} Inhalation anaesthetic) was used to anaesthetise the females prior to milking. Once sedated, the female was given an intramuscular oxytocin injection (Heriot's Oxytocin 10 iu.ml^{-1}, dose rate for bettongs and potoroos: 0.1 ml.kg^{-1}) to initiate the milk let-down. Warm water was used to clean the teat and as an attempt to simulate the sensation of the young attaching to it. Mammary gland and teat were massaged gently if milk would not flow easily.

The milk was collected with a simple milking apparatus (Fig.4.1). It consisted of two pieces of plastic tubing leading to a vacuum chamber, which contained the sampling tube. One piece of tubing was positioned in the investigator's mouth for creating the vacuum in the chamber by suction. The other piece of tubing, which was placed over the appropriate teat, led the milk directly into the sampling tube without contamination with saliva. The sample was subsequently labelled and kept frozen at –20°C until required for analysis. The amount was measured at the time of collection by reading the measurement units on the sampling tube and/or transferring the collected amount with a measuring pipette. Detailed information was recorded for each sample including date, personal ID of mother and young, chosen form of sedation or anaesthetic, (if

appropriate) amount of sedative and time administered, pouch temperature, milk sample number, collected milk amount and teat used for milk collection (Appendix A.4 Data collection sheet).

Fig.4.1: Milk sample collection from an anaesthetised Tasmanian bettong. The upper piece of plastic tubing leads to the investigator's mouth.

After the milk sample collection, the anaesthetic given to the mother was replaced by pure oxygen for her to regain consciousness and muscle control. The young was returned to the location it was found in at capture. Pouch young were returned to the pouch before the mother was completely awake; unfurred young were aided in their reattachment onto the teat. Young-at-foot were either placed back into the nest where they were initially found or released in the cage at the same time with the mother due to their 'following instinct' in the event of her choosing a different nesting site for the rest of the day.

4.2.2 Milk Analysis

The composition of the collected milk samples was analysed for protein, lipid and carbohydrate content as well as for total solids. All milk samples were mixed thoroughly before carrying out any of the assay protocols. To achieve this, samples were defrosted (kept refrigerated at 5°C until completely liquefied), placed in a water bath at 30°C for at least 15 minutes and subsequently

vortexed for 15 seconds using a Whirlimixer (Fisons, UK). The appropriate amount was taken directly afterwards for the respective assay, the rest being refrozen for later use.

4.2.2.1 Protein Analysis

Difficulties were experienced with the determination of the protein content in the collected milk samples, requiring the use of different assays. The protein–dye-binding method (Bradford 1976) was first trialed, but delivered questionable results. The Biuret method (described by Bosset *et al.* 1974, 1974*b*) was then conducted using cow's milk in liquid or powder form as well as milk replacers (Wombaroo, Di-Vetelact) for the test trials. The Biuret method was modified due to lipid interference, which was detected when comparing readings of full cream versus skim milk samples (cow's milk). Several approaches of breaking down the lipid before the actual Biuret reaction proved unsuccessful (addition of NaOH, Na_2EDTA, SDS or Lipase). The combination of Na_2EDTA (Di-Sodium Salt or Ethylenediaminetetra-Acetic Acid, 2%), Lipase (Lipoprotein Lipase Pseudomonas, activity: 33.8 U/MG, ICN, dissolved in a 20mM Tris buffer solution, pH 8.0) and centrifugation of the sample removed the lipid interference and was therefore used for modifying the Biuret method in this project.

The method finally used was as follows: initially the Biuret reagent was prepared by dissolving the respective chemicals in three separate solutions; (a) 1.8g copper sulphate (used as Cupric Sulphate Gran., $CuSO_45H_2O$) in 100ml distilled water, (b) 7.2g potassium sodium tartrate in 300ml distilled water and (c) 36g sodium hydroxide (NaOH) in 400ml distilled water. Solution (a) was added to solution (b) under constant stirring. The resulting mixture was then added to solution (c) and diluted to 1 litre with distilled water. The reagent was very stable over time and did not seem to be affected by sunlight. As a modification of the Biuret method a four-step protocol was employed for the protein analysis, based on the results of the test trials mentioned above.

- In step one of the modified procedure 25µl of undiluted and well-mixed milk were combined with 1µl Lipase (= 1 unit) to break down the triglycerides of the milk into glycerol and fatty acids. The solution was placed in a microcentrifuge tube (QSP, Cat. #: 509-GRD, 1.5ml), mixed for 15 seconds using a vortexer and then kept in a water bath at 37°C for ten minutes. Subsequent to adding chemicals at each step of the procedure, the lid of the microcentrifuge tube was kept closed throughout the procedure to avoid evaporation.

- For step two, 25µl of Na_2EDTA were added to the existing mixture in order to break up the casein micelles in the milk. The mixture was placed on the vortexer for another 15 seconds and then kept for 30 minutes at room temperature.

- In step three, 1.5ml of Biuret reagent were pipetted into the microcentrifuge tube inducing colour formation. The sample was mixed again and placed in a TOMY CAPSULE HF-120 microcentrifuge (TOMY SEIKO CO., LTD., Japan) for 30 minutes at 6400 RPM. This separated the fatty acid precipitates from the rest of the mixture.

- In step four, the transparent solution could then be transferred into a funnel shaped cuvette with a pasteur pipette. The cuvette was placed into a spectrophotometer (SP6-550 UV/VIS spectrophotometer PYE UNICAM) for an absorbency reading at 540 nm. Bovine Serum Albumin (Albumin, Fraction V, USB corporation, Ohio, USA) was used as the standard (Appendix A.6 Standard curves).

If only 5µl of the sample could be utilised for the assay, the same protocol was used with a modification in step two. Only 5µl Na_2EDTA were added to the milk-Lipase solution and 40µl of distilled water were used to adjust the amount accordingly. In this way a reading could be obtained from the standard curve and the appropriate protein amount calculated for the original sample.

4.2.2.2 Carbohydrate Analysis

The carbohydrate content of the milk was measured as total hexose using the phenol-sulphuric acid method (Dubois *et al.* 1956), as modified by Messer and Green (1979) enabling its application to marsupial milk. For this procedure 5µl of well-mixed milk were diluted with 2ml of distilled water and mixed for 15 seconds via vortexing. In a fume cupboard, aliquots of 200µl were transferred into test tubes and combined with 1ml of 3.55% (w/v) phenol solution and 3ml of concentrated sulphuric acid using an autopipette. Directing the acid stream against the liquid surface ensured thorough mixing and maximum heat production. The test tube was shaken by hand for further mixing and left to cool for 30 minutes. The absorbency of the sample was then measured with a spectrophotometer (SP6-550 UV/VIS spectrophotometer PYE UNICAM) at 490 nm. Milk Sugar α-Lactose (SIGMA L3625) was used as a standard (Appendix A.6 Standard curves).

4.2.2.3 Lipid Analysis

The lipid content of the collected milk samples was determined using the creamatocrit method (Lucas *et al.* 1978), which is based on a micro centrifugation method described by Fleet and Linzell (1964). Depending on the available sample amount, up to 75µl of milk were drawn by capillary action into a microhaematocrit tube (BRAND, length: 75mm ± 1mm, inner diameter: 1.1-1.2mm, outer diameter: 1.5-1.6mm). The tube was then flame sealed at the opposite end to avoid burning of milk. Great care was taken to constantly turn it in the flame to prevent the glass from cooling down on an angle, resulting in a deformed meniscus.

The tubes were then placed in a centrifuge (MSE centrifuge 'Minor' with haematocrit attachment, cycles: 50/60) operated on slow speed (setting 1) for the first five minutes, followed by maximum speed (setting 10) for one hour to ensure a clear separation of layers within the sample. The cream layer separated at the top of the column in most cases with a liquid fat layer above. The tubes were placed vertically in plasticine immediately after the centrifuge had stopped to

prevent the layers from setting at an angle. The lengths of the cream and liquid fat layer combined as well as the total length of the milk column were measured to the top of the meniscus using a haematocrit reader (MSE Micro-haematocrit Reader). Readings were obtained within the 30 minutes following centrifugation to avoid inaccuracy due to blending of layers with time. The creamatocrit (%) was then calculated as follows:

$$\text{creamatocrit}\,(\%) = \frac{\text{length of fat layer (cream and liquid fat)}}{\text{total length of milk column}} \times 100$$

The Roese-Gottlieb ether extraction technique (cited in Horwitz 1980) was implemented for standardising the creamatocrit method. A linear relationship is expressed between the fat concentration measured by the Roese-Gottlieb method and the creamatocrit, enabling the calculation of lipid ($g.100ml^{-1}$ milk) for both species using the following equations (Appendix A.6 Standard curves):

$$\text{lipid}\,(g.100ml^{-1}\,\text{bettong milk}) = \frac{(\text{creamatocrit} + 1.4647)}{1.0871}$$

$$\text{lipid}\,(g.100ml^{-1}\,\text{potoroo milk}) = \frac{(\text{creamatocrit} + 0.312)}{1.037}$$

4.2.2.4 Total Solids

Depending on availability, approximately 50µl of milk were pipetted into pre-weighed microcentrifuge tubes and re-weighed. The samples were then placed for 24 hours in a 100°C fan forced oven (Laboratory Oven, Thermoline L+M, Australia) and dried to a constant weight. For transport purposes, samples were kept in a desiccator containing dehydrated silica gel to prevent a weight gain due to humidity absorption through the air. They were re-weighed after 48 hours in the oven to ensure that no weight change had occurred. All weight measurements were obtained to the nearest 1µg using a Mettler balance (AE100, Switzerland). The total amount of solids in the milk sample was calculated using the following equation:

$$\text{solids (\%)} = \frac{\text{dry weight}}{\text{wet weight}} \times 100$$

4.2.2.5 Energy content

The energy content of the milk was calculated using the following conversion factors: 16.5kJ.g^{-1} for milk carbohydrates, 24.6kJ.g^{-1} for protein and 38.1kJ.g^{-1} for lipid as outlined by Rose *et al.* 2003.

4.2.2.6 Re-use of Milk

The amount of the collected milk samples differed greatly over lactation. Some samples did not provide sufficient amounts for covering all the assays described above. Since the composition of milk was not changed by centrifugation, it was decided to collect small samples for re-use purposes after completing the lipid assay (4.2.2.3 Lipid Analysis). Instead of being discarded, the micro-haematocrit tubes were sealed with plasticine (seal-ease®) at the open end. They were then placed back into the freezer (-20°C) to await further analysis.

It was not possible to mix the layered milk within the haematocrit tube. Before re-use, the samples needed to be transferred into a bigger device in which they could be thoroughly mixed. Several approaches of blowing the contents out of the tube were trialed in cooperation with the Chemistry department. They proved to be unsuccessful and were based on an attachment to the haematocrit tube (a) an inflation bulb and b) a hypodermic needle with tubing attached to blow the content out of the tube either under pressure or by mouth, using cotton material within the tube for saliva protection). Subsequently it was decided to push the sample out of the haematocrit tube. For removal of the sealed ends, the tube was placed into a v-shaped device to provide enough support during the cutting process and avoid breaking the entire tube. Both ends, plasticine filled and flame-sealed, were broken off. Where the break was anticipated, the glass was first scratched with a Tungsten Carbide blade and then broken off by a quick tap with the blade. A glass rod was custom made by a glassblower, which exactly fitted into the haematocrit tube. This was inserted at the former flame sealed end of the tube, using the thick layer of solids to push the content

through the haematocrit tube into a microcentrifuge tube. The frozen state of the sample was beneficial for removing the complete content without leaving any residue on the tube wall. The recovered sample could then be placed into the vortexer for remixing the milk.

4.2.2.7 Statistical Analysis

A One-Way-ANOVA with subsequent post-hoc comparison (Least Significant Difference t-Test, 0.05 level of probability) was performed for detecting significant differences between the milk samples produced for different transfer categories (cross-foster, foster and original young for both species) as well as TADs per category. Tests were conducted for each individual week of lactation given as associated 'pouch age' of the lactating female.

4.3 Results

A total of 460 milk samples was collected from 31 bettong females (340 milk samples) and 13 potoroo females (120 milk samples). The individual milk assay results are presented for both bettong and potoroo data to facilitate easier cross-species comparisons. Pouch age refers to the age of the young, which initially inhabited the pouch and therefore does not necessarily represent the age of the present young, if it is a transfer young. Pouch age is meant to be a guideline for the age of the milk sample rather than the young drinking it. All results (unless indicated otherwise) are presented as mean (M) ± standard error (SE). In the graph description 'N' is provided as the number of individuals/ datapoints.

4.3.1 Milk volume

The milk volume values for bettong milk (Fig.4.2) and potoroo milk (Fig.4.3) both described an inverse U-shaped relation between the two variables, volume and pouch age. The bettong milk volume reached maximum levels at pouch age week 17 for cross-foster young (1.42ml ± 0.14) and week 18 for foster young (1.31ml ± 0.14). Although the samples for original young did not follow the same clear pattern, they still reached an equally high peak level at week 12 (1.37ml ± 0.09), preceding the pouch vacation of the bettong, usually occurring

at week 15. The milk volume decreased to near zero at week 26 for foster young, week 29 for cross-foster young and week 34 for original young. Volumes for both cross-foster and original young became subject of fluctuation from week 26 until weaning.

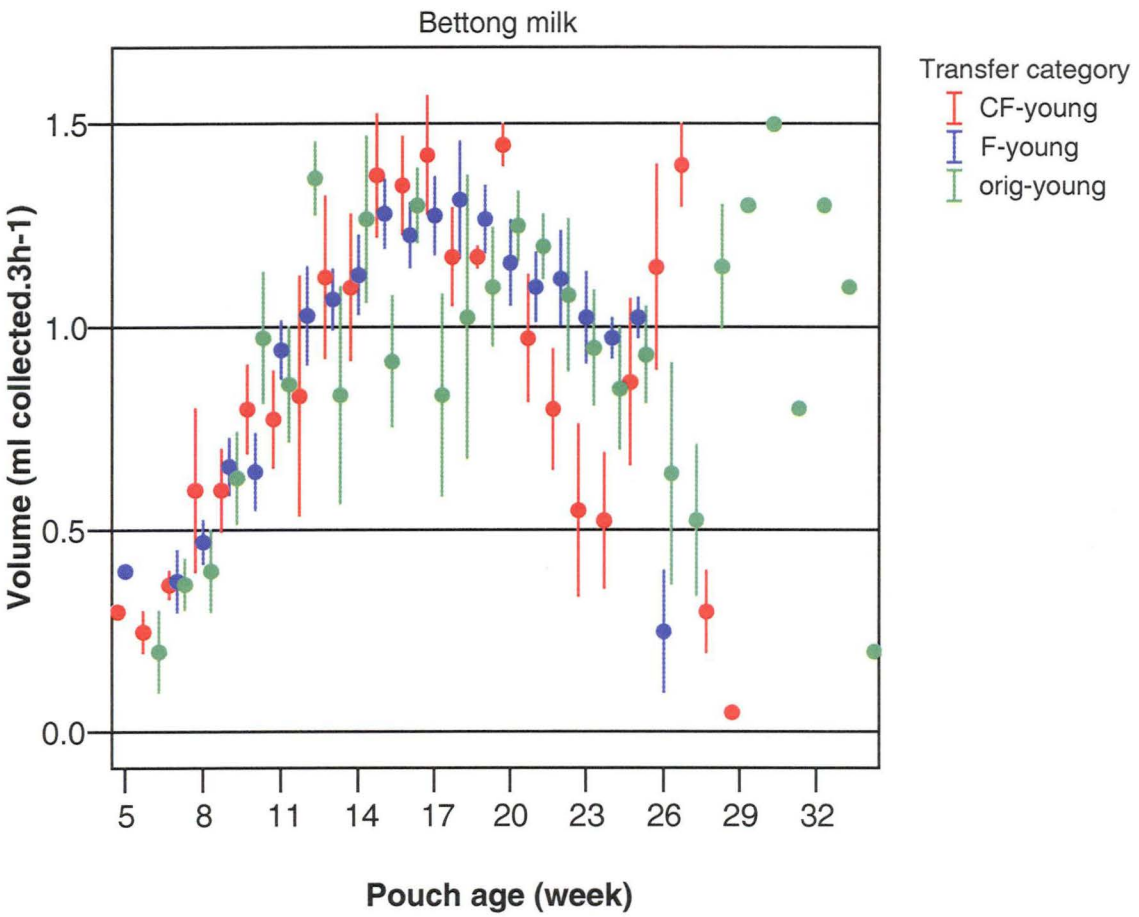

Fig.4.2: Changes in volume (ml collected.3h^{-1}) of bettong milk produced for cross-foster young (CF, red, potoroo, N=4/81), foster young (F, blue, bettong, N=12/140) and original young (orig, green, bettong, N=15/119) throughout lactation. Error bars show Mean ± 1.0 SE.

Although there appeared to be little difference in mean volume between the transfer groups throughout lactation, statistical analysis indicated a significant difference in late lactation for pouch age week 27 [$F_{(1, 4)}$=9.552, p=0.037] and week 28 [$F_{(1, 2)}$=22.231, p=0.042]. *Post-hoc* comparison using the LSD-test showed that the mean volume for cross-foster young (1.40ml ± 0.10) was significantly larger than for original young (0.53ml ± 0.18) in week 27 and vice versa for week 28 (orig: 1.15ml ± 0.15, CF: 0.30ml ± 0.10).

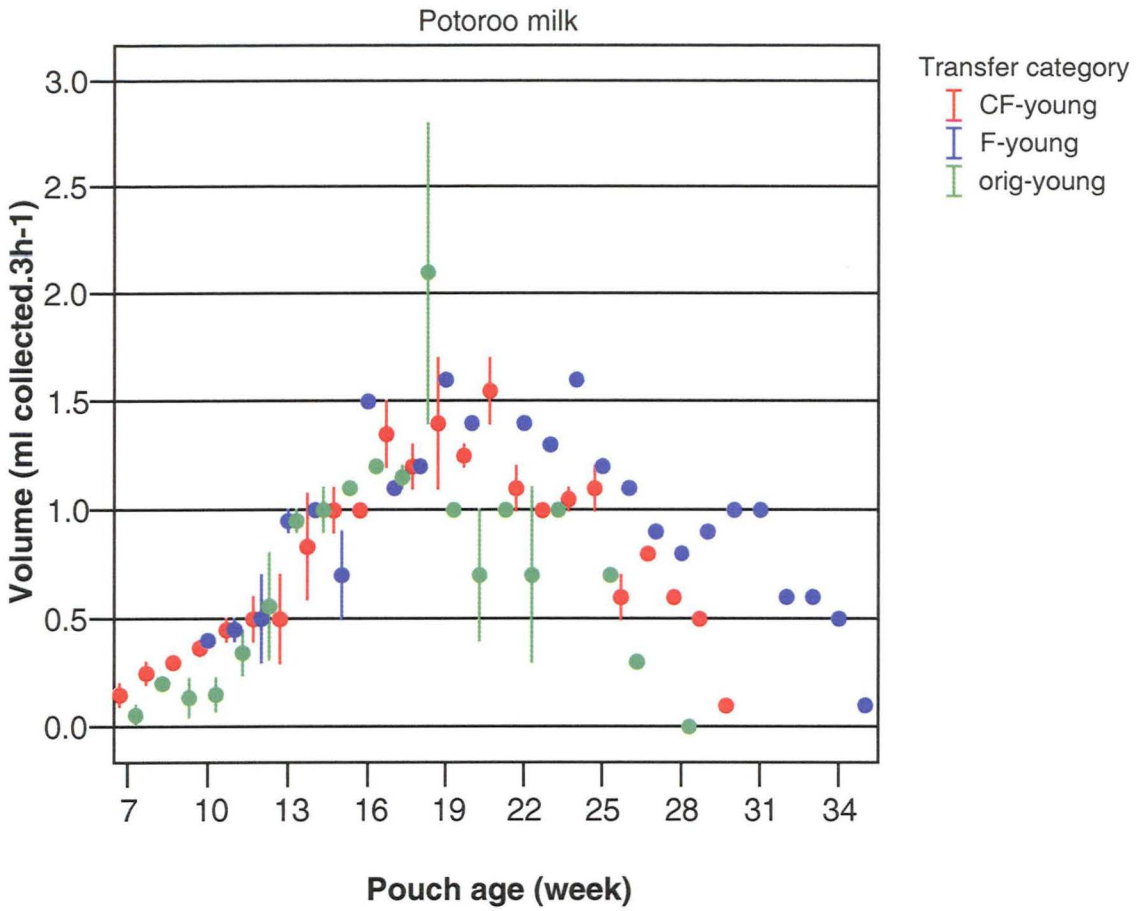

Fig.4.3: Changes in volume (ml collected.3h^{-1}) of potoroo milk produced for cross-foster young (CF, red, bettong, N=4/50), foster young (F, blue, potoroo, N=2/31) and original young (orig, green, potoroo, N=7/38) throughout lactation. Error bars show Mean ± 1.0 SE.

Although the slopes of the described U-shape appeared to be less steep for the potoroo milk volumes, maximum levels were reached close to pouch vacation of the potoroo young, usually occurring at pouch age week 17 (Fig.4.3). A mean maximum volume of 2.1ml was produced for original young at week 18. Peak levels for foster young were reached at 1.6ml (week 19) whereas an equal amount was produced for cross-foster young at week 21. The end of lactation (with volumes close to zero) was reached for original young at week 28, for cross-foster young at week 30 and foster young at week 35.

The volumes for original young in comparison with the other two transfer categories appeared to increase more slowly in early lactation and decrease more rapidly towards weaning. A statistically significant difference at the 5% level of probability was detected for early lactation (week 10) [$F_{(2, 5)}$=6.031, p=0.046]. The mean volume for original young (0.15ml ± 0.08) was significantly smaller compared to cross-foster (0.37ml ± 0.03) and foster young (0.40ml ± 0.00). The latter two did not differ significantly from each other.

4.3.2 Carbohydrate

Carbohydrate concentrations in both bettong (Fig.4.4) and potoroo milk samples (Fig.4.5) showed an increase in early lactation. Peak concentrations were reached just before pouch vacation and were followed by a decrease of carbohydrate content with age.

The bettong milk samples showed some fluctuation in early lactation with initial carbohydrate concentrations ranging from 6.40g.100ml^{-1} for foster young and 10.80g.100ml^{-1} for cross-foster young (both week 5) to 12.06g.100ml^{-1} ± 1.40 for original young (week 6). A significant difference was detected for week 8 [$F_{(2, 8)}$=7.857, p=0.013], when mean carbohydrate concentration for foster young (13.79g.100ml^{-1} ± 0.34) were significantly different from original young (10.15 g.100ml^{-1} ± 1.02) and cross-foster young (9.75g.100ml^{-1} ± 2.49). The latter two were not significantly different from each other.

Peak levels for the two transfer categories were reached at pouch age week 11 (cross-foster young: 13.55g.100ml^{-1} ± 0.59 and foster young: 14.29g.100ml^{-1} ± 0.56) and week 13 (original young: 14.22g.100ml^{-1} ± 3.34). This was followed by a rapid decrease in concentration to ca. 3g.100ml^{-1} in week 19 for all three transfer categories with a subsequent further decline to values near zero over the next ten weeks of lactation.

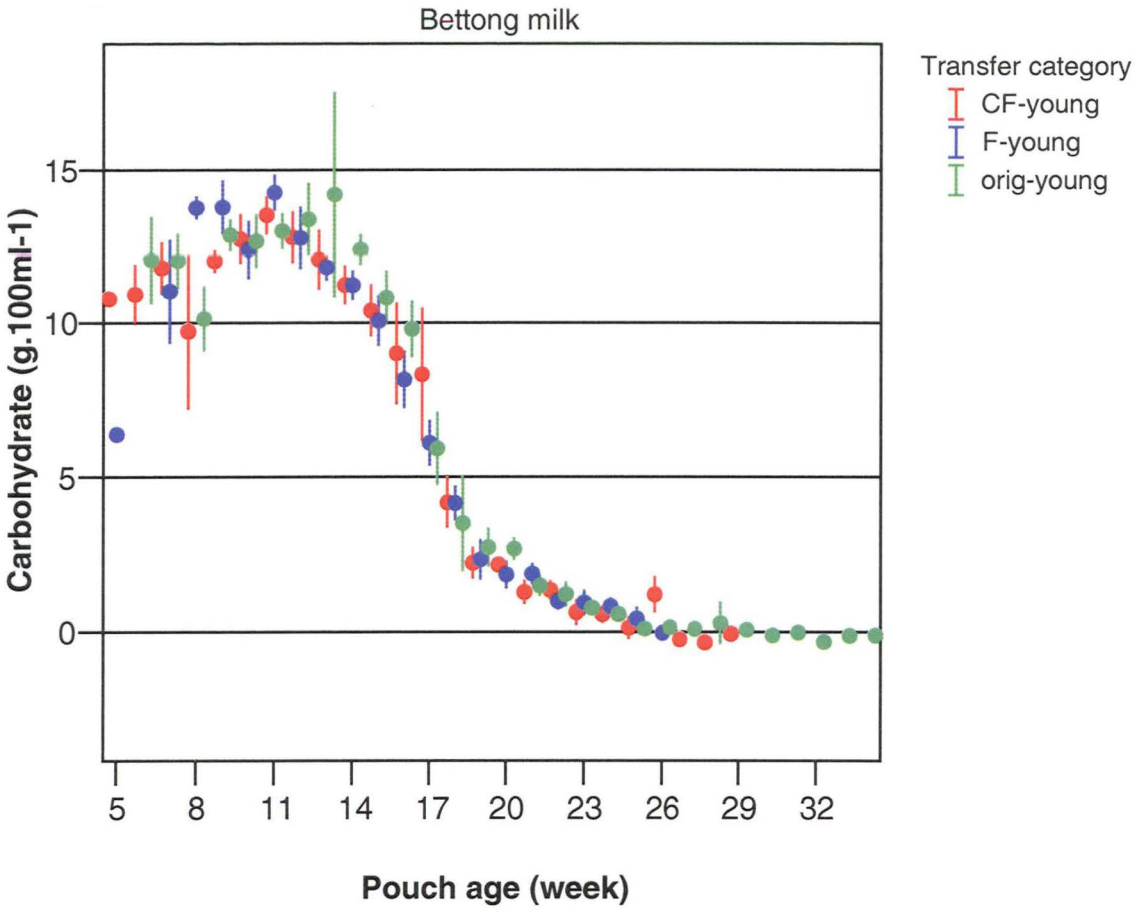

Fig.4.4: Changes in carbohydrate content (g.100ml^{-1}) of bettong milk produced for cross-foster young (CF, red, potoroo, N=4/80), foster young (F, blue, bettong, N=12/140) and original young (orig, green, bettong, N=15/118) during lactation. Error bars show Mean ± 1.0 SE.

Initial carbohydrate concentrations in potoroo milk ranged from ca. 9g.100ml^{-1} for cross-foster young and original young (both week 7) to 13.67g.100ml^{-1} ± 0.38 for foster young (week 10) (Fig.4.5). Maximum levels were reached for the latter two at week 12 (orig-young: 13.92g.100ml^{-1} ± 0.63, F-young: 15.01 g.100ml^{-1} ± 0.61), while potoroo milk for cross-foster young provided peak concentrations in week 15 (13.90g.100ml^{-1} ± 2.46). Like the bettong milk, potoroo carbohydrate concentrations rapidly decreased to ca. 3g.100ml^{-1} at week 22 for all three transfer categories and eventually reached near zero values within the next ten to twelve weeks of lactation. Although there was much greater fluctuation in early potoroo lactation, significant differences between the transfer groups were found in late lactation (week 24 [$F_{(1,\ 1)}$=201.720, p=0.045] and week 26 [$F_{(2,\ 1)}$=212.028, p=0.049]).

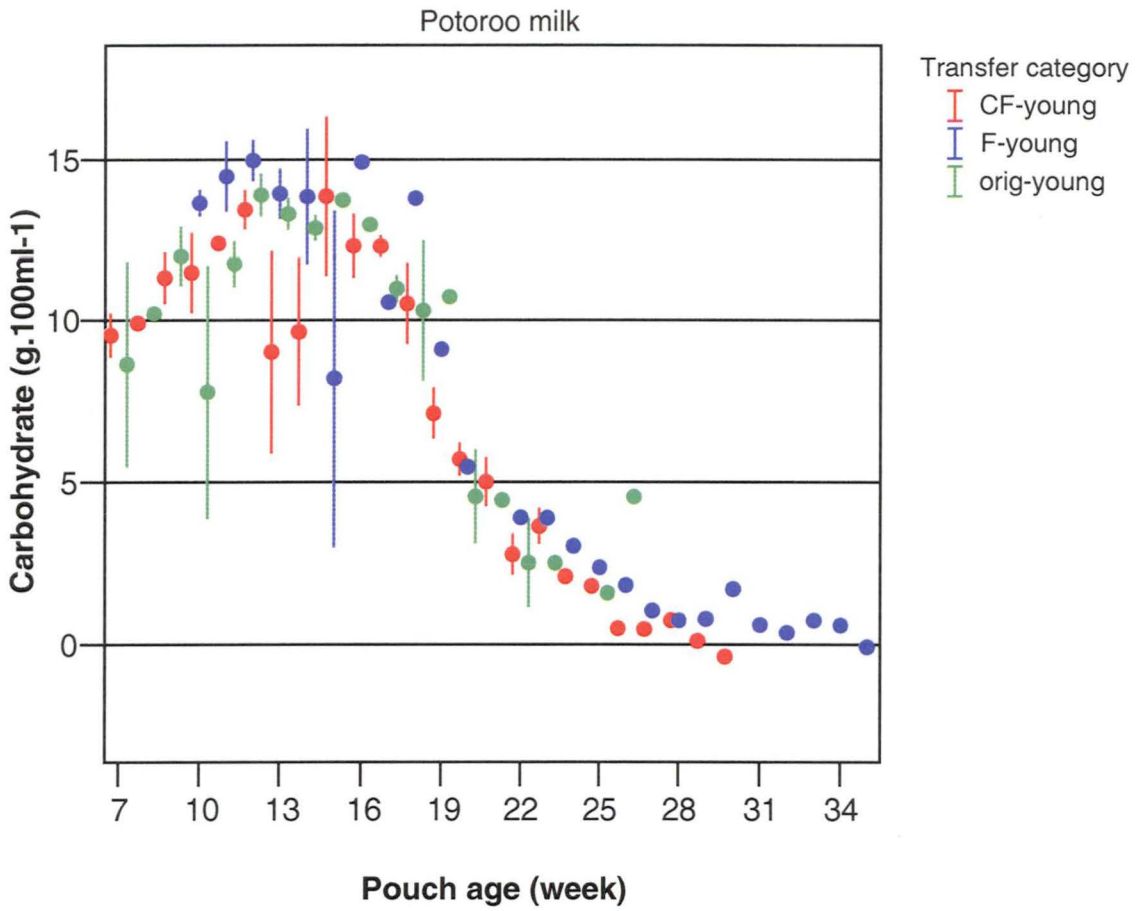

Fig.4.5: Changes in carbohydrate content (g.100ml^{-1}) of potoroo milk produced for cross-foster young (CF, red, bettong, N=4/50), foster young (F, blue, potoroo, N=2/31) and original young (orig, green, potoroo, N=7/35) during lactation. Error bars show Mean ± 1.0 SE.

4.3.3 Lipid

The lipid concentration in the milk samples of both species underwent considerable changes through lactation. Concentrations remained low for most of early and mid-lactation, followed by a strong increase just before pouch vacation. Maximum levels were reached in late lactation, followed by a rapid decline towards weaning.

Lipid concentrations in the bettong milk (Fig.4.6) remained low in early lactation for all three transfer categories (between 3 and 5g.100ml^{-1} until week 11). This plateau phase was followed by a rapid increase in concentration with maximum lipid levels being reached in week 21 for cross-foster young (26.03g.100ml^{-1} ± 3.84), week 22 for foster young (20.85g.100ml^{-1} ± 3.10) and week 23 for original

young (20.26g.100ml^{-1} ± 3.71). Subsequently all values declined to near zero towards the end of lactation (F-young: week 26, CF-young: week 28, orig-young: week 34).

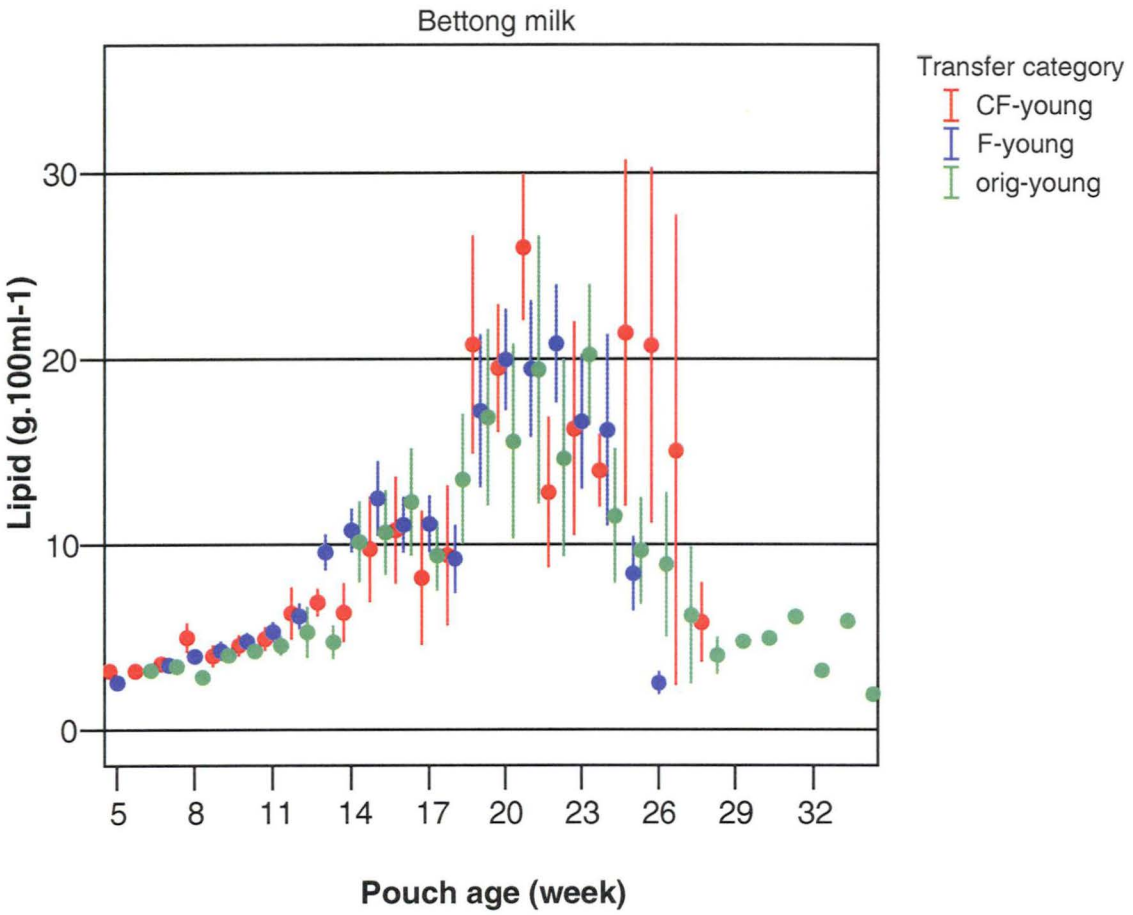

Fig.4.6: Changes in lipid content (g.100ml^{-1}) of bettong milk produced for cross-foster young (CF, red, potoroo, N=4/77), foster young (F, blue, bettong, N=12/140) and original young (orig, green, bettong, N=15/112) throughout lactation. Error bars show Mean ± 1.0 SE.

There was a considerable amount of fluctuation in the lipid values following the initial plateau phase. A significant difference between the transfer categories was identified in week 13 [$F_{(2,\ 14)}$=5.092, p=0.022]. *Post-hoc* comparisons showed that the mean lipid concentration for original young (4.76g.100ml^{-1} ± 0.83) was significantly lower than for foster young (9.60g.100ml^{-1} ± 0.91).

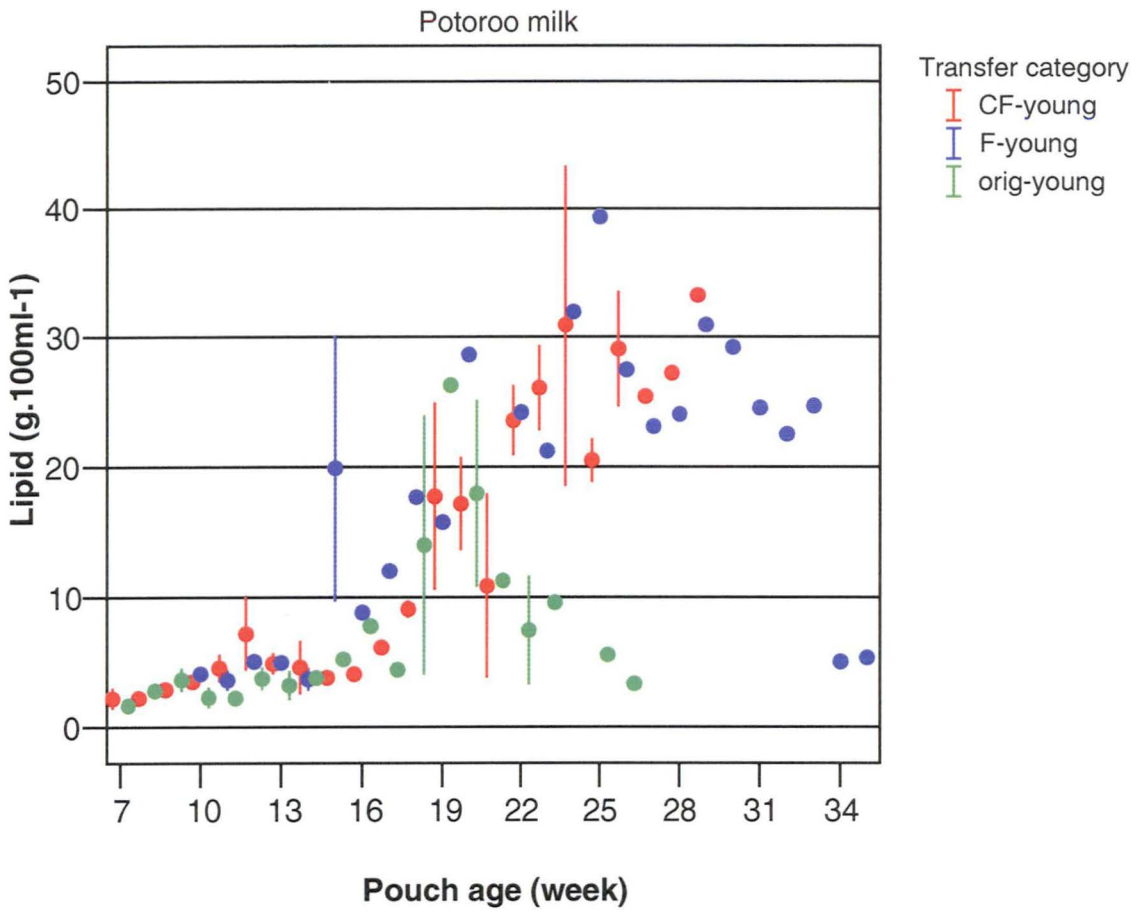

Fig.4.7: Changes in lipid content (g.100ml^{-1}) of potoroo milk produced for cross-foster young (CF, red, bettong, N=4/48), foster young (F, blue, potoroo, N=2/31) and original young (orig, green, potoroo, N=5/32) throughout lactation. Error bars show Mean ± 1.0 SE.

The same distinct plateau phase was apparent in early and mid-lactation for potoroo milk samples (Fig.4.7). Lipid remained initially at about 3g.100ml^{-1} for all three transfer categories. The rapid increase in concentration occurred at week 15 and resulted in maximum lipid concentrations of 26.31g.100ml^{-1} for original young (week 19), 39.38g.100ml^{-1} for foster young (week 25) and 33.29 g.100ml^{-1} for cross-foster young (week 29). Corresponding with the delay in reaching peak lipid levels, a decrease in values to near zero occurred much later for the fostered young (week 35) compared to original young (week 26). The increase and subsequent decline in lipid concentration appeared to be less synchronised within the transfer categories. A significant difference between the groups was found in week 16 [$F_{(2,\ 1)}$=28729.672, p=0.004] and week 17 [$F_{(2,\ 2)}$=104.012, p=0.010].

4.3.4 Protein

Protein concentrations increased with pouch age in both bettong (Fig.4.8) and potoroo milk (Fig.4.9). Maximum levels were reached in late lactation with concentrations decreasing towards weaning. The protein concentration in bettong milk remained mostly between 7 and 10g.100ml^{-1} in early lactation for foster and cross-foster young. Values began to increase at week 12, forming an initial peak at week 14 just before pouch vacation (CF-young: 11.42g.100ml^{-1} ± 0.20, F-young: 12.14g.100ml^{-1} ± 0.40). Protein concentrations reached maximum levels in week 22 for both foster young (16.45g.100ml^{-1} ± 0.69) and cross-foster young (16.02g.100ml^{-1} ± 0.14).

Protein concentrations for original young did not remain constant, but increased from ca. 6g.100ml^{-1} at week 6 to over 18g.100ml^{-1} at week 21 (mean protein concentration at first peak, week 14, 13.03g.100ml^{-1} ± 0.87). Significant differences in mean protein concentration between the transfer groups were found in week 8 [$F_{(2, 8)}$=5.614, p=0.030] and week 26 [$F_{(2, 3)}$=11.250, p=0.040]. The protein content in samples for cross-foster young (10.27g.100ml^{-1} ± 1.97) in week 8 was higher compared to samples for foster (7.40g.100ml^{-1} ± 0.33) and original young (6.43g.100ml^{-1} ± 0.07). The latter two did not differ significantly from each other.

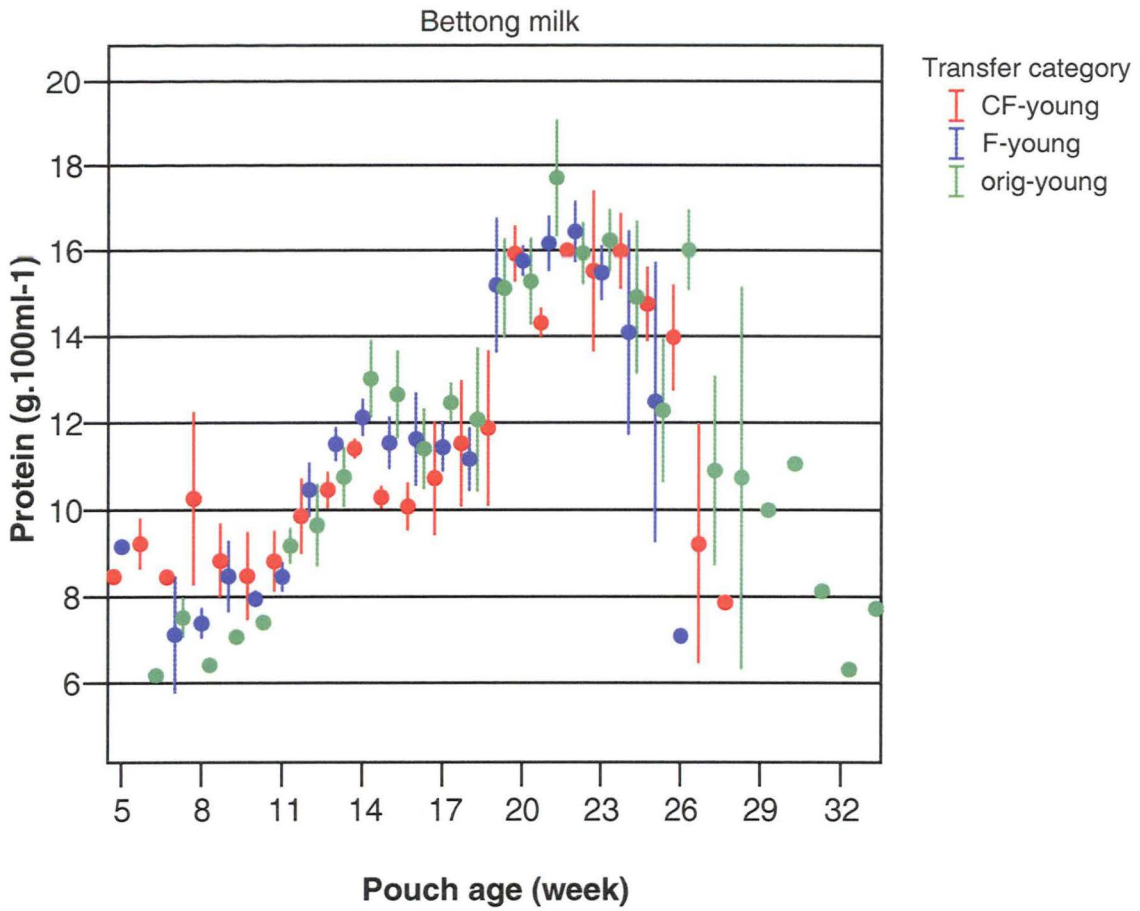

Fig.4.8: Changes in protein content ($g.100ml^{-1}$) of bettong milk produced for cross-foster young (CF, red, potoroo, N=4/76), foster young (F, blue, bettong, N=12/136) and original young (orig, green, bettong, N=15/107) during lactation. Error bars show Mean ± 1.0 SE.

The results for protein concentrations in potoroo milk (Fig.4.9) followed the above described trend, but did not form a pronounced initial peak around pouch vacation. The plateau phase lasted until week 14 with protein concentrations of ca. $7g.100ml^{-1}$ for all three transfer categories. The subsequent increase in protein levels reached a slightly higher maximum for potoroo milk samples with mean values ranging from ca. $16g.100ml^{-1}$ (orig-young: week 25) to ca. $23g.100ml^{-1}$ (CF-young: week 27).

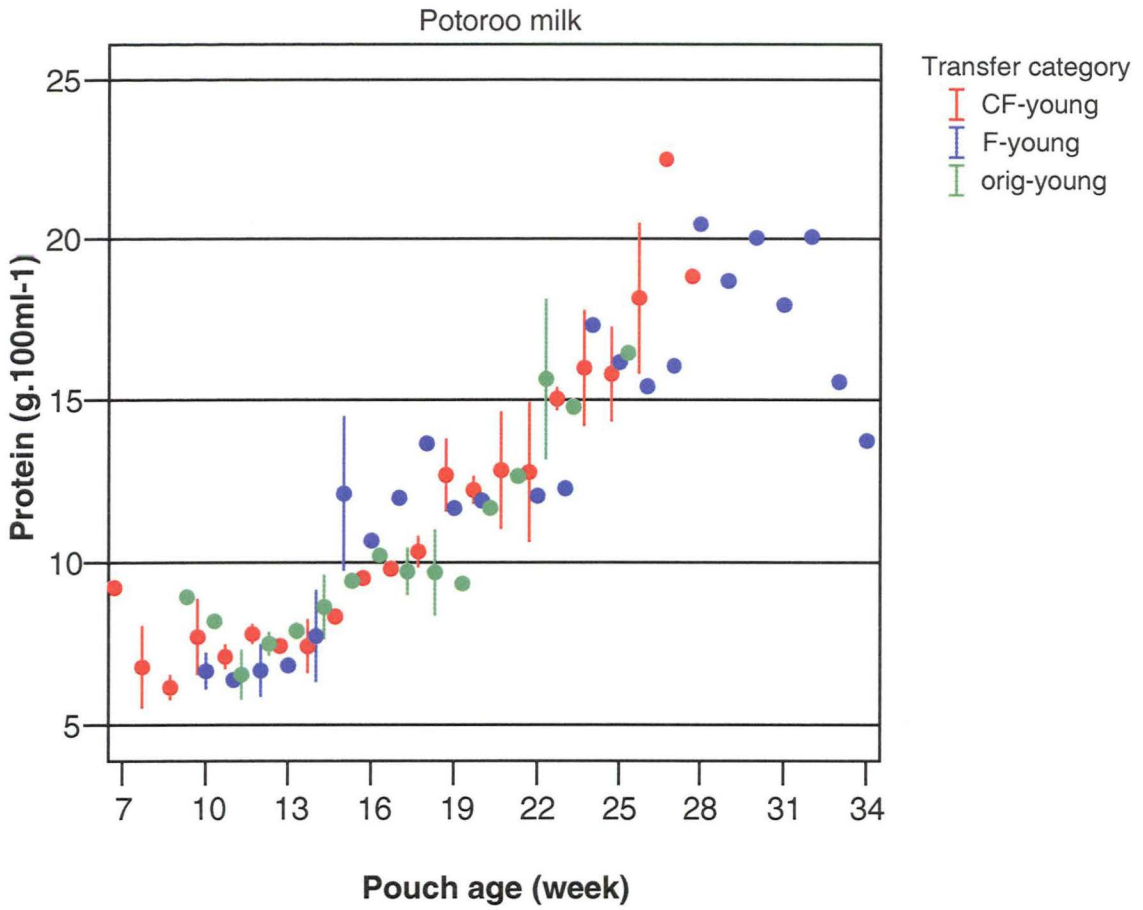

Fig.4.9: Changes in protein content (g.100ml^{-1}) of potoroo milk produced for cross-foster young (CF, red, bettong, N=3/45), foster young (F, blue, potoroo, N=2/30) and original young (orig, green, potoroo, N=4/26) during lactation. Error bars show Mean ± 1.0 SE.

Milk samples produced for foster young reached a protein peak with just over 20g.100ml^{-1} at week 28 and demonstrated the subsequent decrease of concentration towards weaning. The decline is not evident in the other two transfer categories, most probably due to small sample sizes and therefore insufficient milk for all assays. Significant differences in mean protein concentration between the transfer groups were found for week 13 [$F_{(2, 4)}$= 8.085, p=0.039] and week 16 [$F_{(2, 1)}$=288.000, p=0.042]. The mean protein content in samples for original young (7.92g.100ml^{-1} ± 0.215) in week 13 was significantly higher than in samples for foster young (6.86 g.100ml^{-1} ± 0.01).

4.3.5 Total Solids

The solid contents of the bettong milk samples (Fig.4.10) described a two peak trend with a subsequent decrease towards weaning. This trend was less pronounced for the total solids of the potoroo milk samples (Fig.4.11), which fluctuated greatly in late lactation.

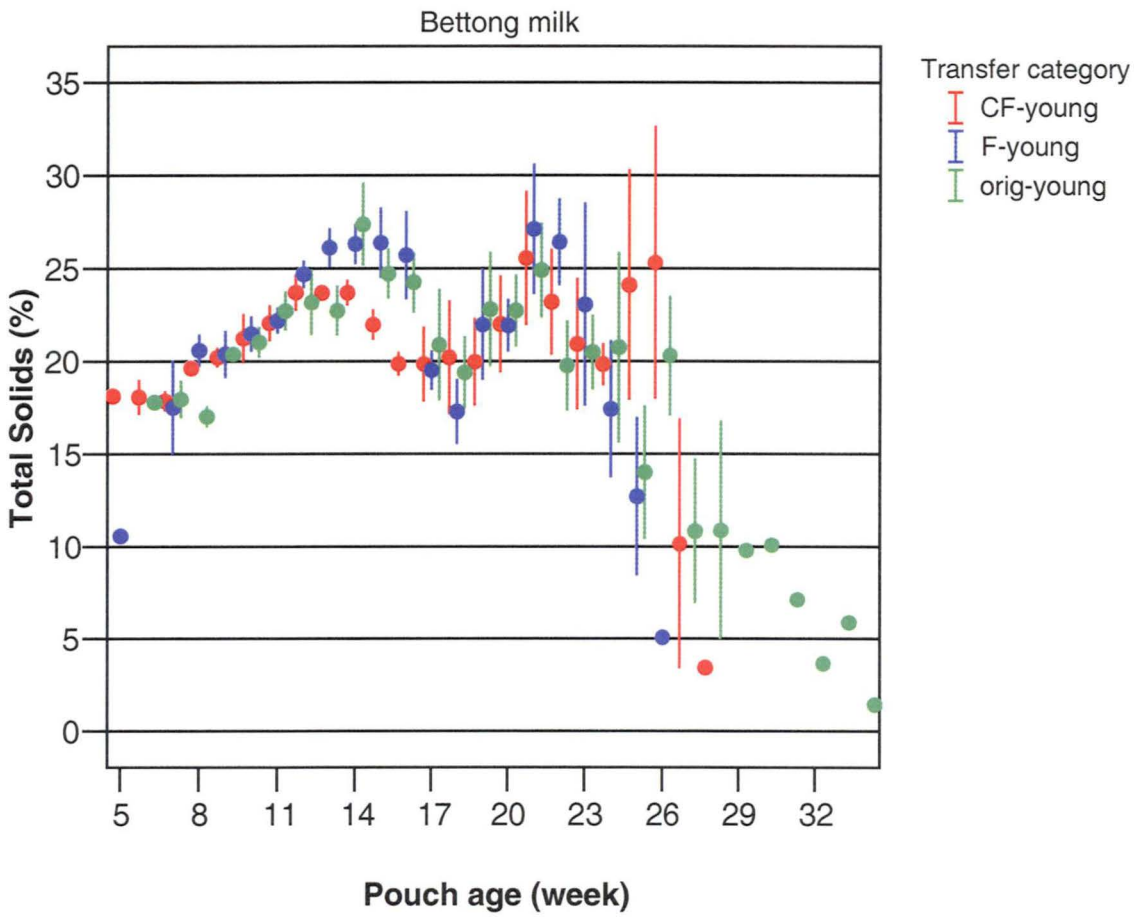

Fig.4.10: Changes in total solid content (%) of bettong milk produced for cross-foster young (CF, red, potoroo, N=4/76), foster young (F, blue, bettong, N=12/136) and original young (orig, green, bettong, N=15/107) throughout lactation. Error bars show Mean ± 1.0 SE.

The was a clear trend in the solid content of the bettong milk for all three transfer categories with values of ca. 18% (week 7) rising to levels between 24% (CF) and 27% (orig) in week 14 just before pouch vacation. They decreased to their initial range only to form a second peak of similar proportion at week 21 with a subsequent decline of total solids close to zero at weaning (F-young: week 26, CF-young: week 28, orig-young: week 34). There was greater fluc-

tuation in the values towards weaning, but no significant differences in mean solids content between the transfer groups occurred at any stage of lactation.

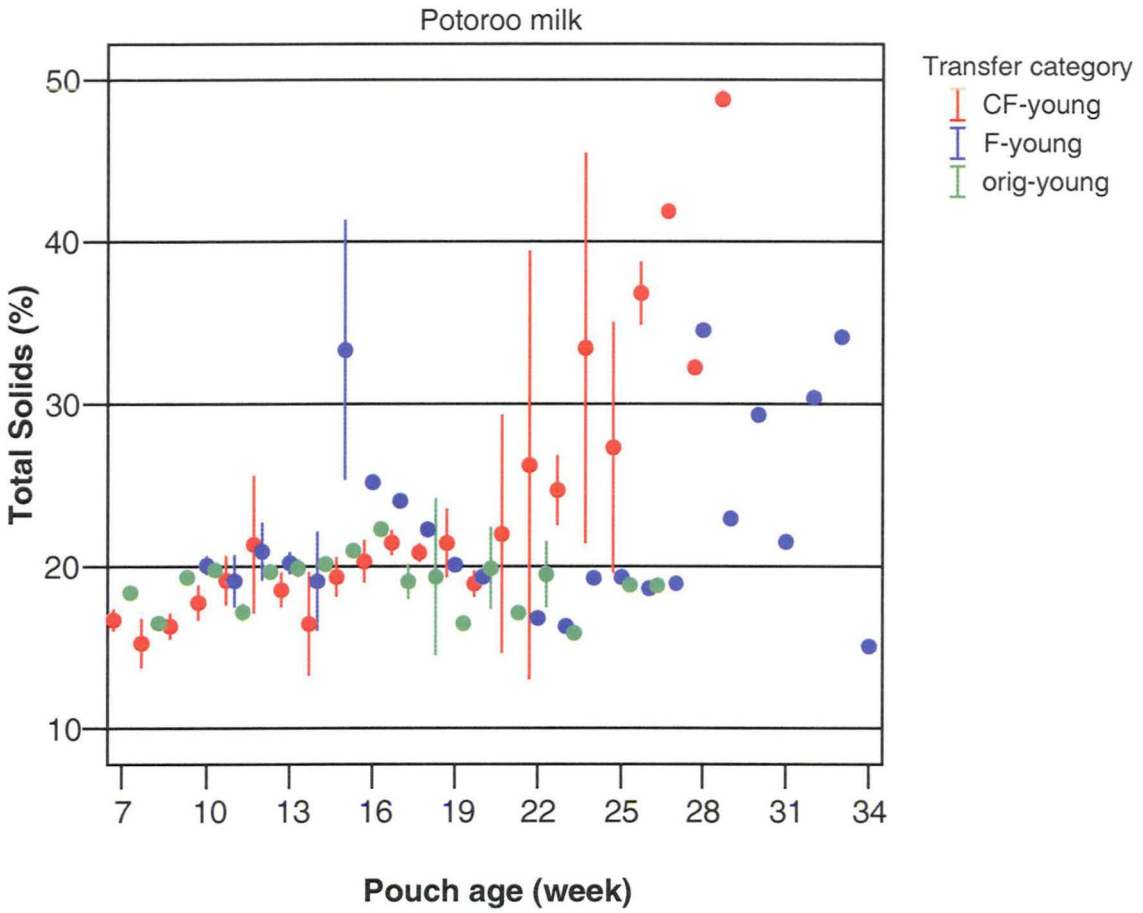

Fig.4.11: Changes in total solid content (%) of potoroo milk produced for cross-foster young (CF, red, bettong, N=3/47), foster young (F, blue, potoroo, N=2/30) and original young (orig, green, potoroo, N=4/29) throughout lactation. Error bars show Mean ± 1.0 SE.

The solid content of the potoroo milk samples oscillated around 20% in early and mid-lactation for original and cross-foster young. Total milk solids for foster young represented an exception, with a pronounced peak of 33.35% ± 7.93 at week 15 just before pouch vacation and a subsequent decrease to the 20% level by week 19. Milk samples produced for cross-foster and foster young showed high levels of fluctuation in solid content towards weaning. The absence of a decline in total solid concentration in late lactation is most probably due to small sample sizes and therefore insufficient milk for all assays as seen in protein concentrations.

4.3.6 Energy content

The energy content of bettong (Fig.4.12) and potoroo milk (Fig.4.13) increased with pouch age. Maximum levels were reached in late lactation with a subsequent decrease towards weaning.

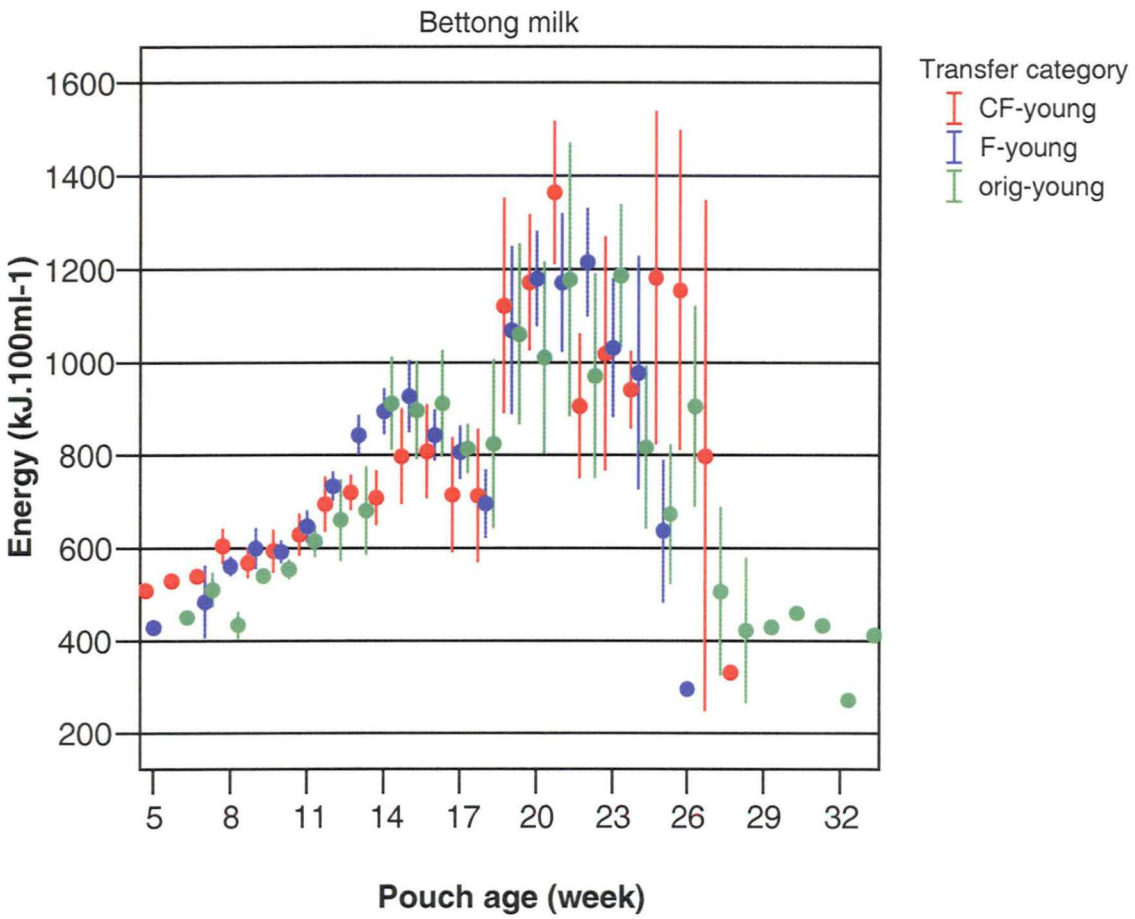

Fig.4.12: Changes in energy content (kJ.100ml^{-1}) of bettong milk produced for cross-foster young (CF, red, potoroo, N=4/76), foster young (F, blue, bettong, N=12/136) and original young (orig, green, bettong, N=15/107) during lactation. Error bars show Mean ± 1.0 SE.

The energy content for cross-foster young showed a steady increase from week 5 (M=509.68kJ.100ml^{-1}) to week 16 (809.24kJ.100ml^{-1} ± 98.87), followed by a rapid increase, which resulted in maximum energy levels at week 21 (1365.91 kJ.100ml^{-1} ± 150.83). Values for foster young (M=429.44kJ.100ml^{-1}, week 5) and original young (M=451.45kJ.100ml^{-1}, week 6) increased more rapidly in early lactation and formed an initial peak just over 900kJ.100ml^{-1} at week 14 (original young) and week 15 respectively (foster young) before pouch vacation.

Maximum energy concentrations were produced at week 22 for foster young (1215.77kJ.100ml^{-1} ± 113.49) and week 23 for original young (1186.93 kJ.100ml^{-1} ± 150.50).

Fluctuation in energy levels increased with pouch age for all three transfer categories. Significant differences between the groups were detected in early lactation (week 6 [$F_{(1, 1)}$=6383.530, p=0.008], week 8 [$F_{(2, 8)}$=7.797, p=0.013]). The mean energy content in samples for original young (434.80kJ.100ml^{-1} ± 26.26) in week 8 was significantly lower than in samples for cross-foster young (605.04kJ.100ml^{-1} ± 35.13) and foster young (561.82kJ.100ml^{-1} ± 17.80). The latter two categories did not differ significantly from each other.

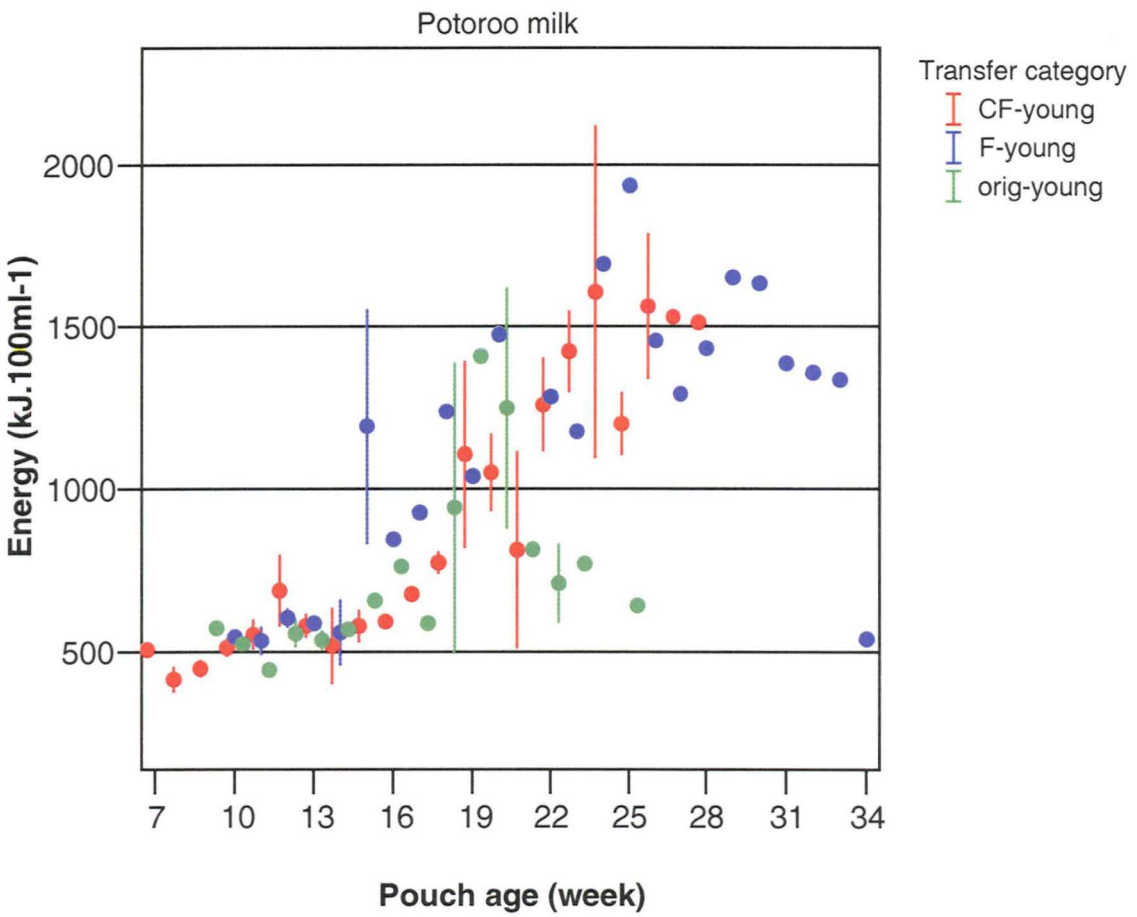

Fig.4.13: Changes in energy content (kJ.100ml^{-1}) of potoroo milk produced for cross-foster young (CF, red, bettong, N=3/45), foster young (F, blue, potoroo, N=2/30) and original young (orig, green, potoroo, N=4/26) during lactation. Error bars show Mean ± 1.0 SE.

The energy content of potoroo milk (Fig.4.13) remained constant at ca. 500kJ.100ml^{-1} in early lactation, with a rapid increase thereafter. The milk samples produced for original young had maximum energy levels of just over 1400kJ.100ml^{-1} in week 19. Peak concentrations for cross-foster and foster young were higher (CF-young: 1609.18kJ.100ml^{-1} ± 511.59, F-young: M= 1937.64kJ.100ml^{-1}), but were also reached later in lactation (CF-young: week 24, F-young: week 25). There was considerable fluctuation in energy concentrations during mid and late lactation for all transfer categories. A significant difference between the groups was found for week 17 [$F_{(2, 2)}$=174.852, p=0.006].

4.3.7 Milk composition in relation to transfer age difference

The above presented results were based on grouped transfer categories with young of different ages drinking the milk of the same 'pouch age' or lactation week. Both categories for foster and cross-foster young needed to be examined to the weekly level of TAD to ensure that the combining of age classes did not mask an underlying difference between the groups. The milk sample results for transferred young were examined in two stages. Firstly, the milk results for transfer young were split into general age difference (same age, younger or older than the original young, which used to inhabit their pouch). In a second step the same dataset was divided further into the TAD in weeks, ranging from three weeks younger (-3) in weekly intervals to three weeks older (3). Since a limited amount of information was gained from every individual graph, only the energy content is presented exemplary for the bettong milk samples produced for foster young (Fig.4.14).

When splitting each transfer category into general age difference groups, a significant differences in mean energy was found in bettong milk for foster young at time of pouch vacation (week 15 [$F_{(2, 7)}$=6.832, p=0.023]). The energy content in samples produced for younger foster bettong young was higher (1163.45 kJ.100ml^{-1} ± 150.50) than in samples for older foster young (725.54kJ.100ml^{-1} ± 46.60). Values for same aged foster young did not differ significantly from results of either younger or older foster young.

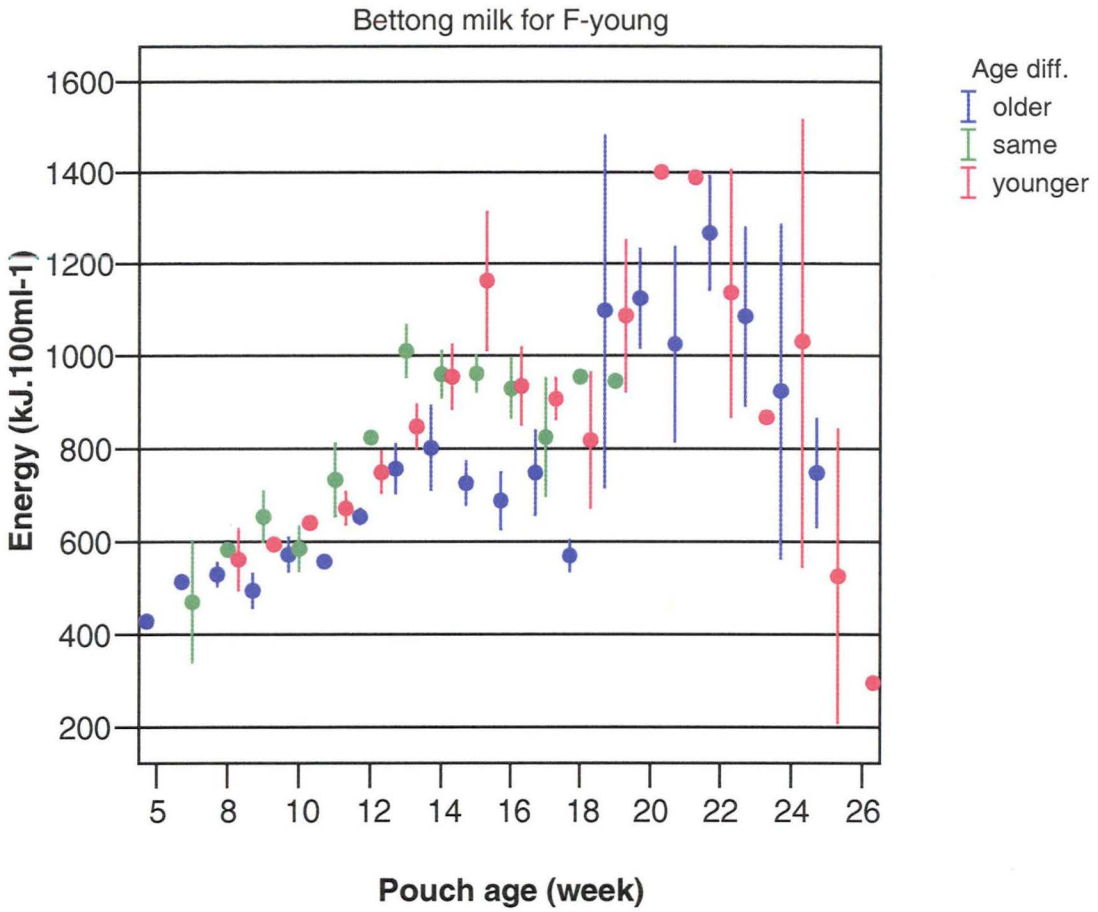

Fig.4.14: Changes in energy content (kJ.100ml^{-1}) of bettong milk produced for foster young (bettong) of older (blue, N=5/61), same (green, N=4/31) or younger age (purple, N=4/44) than the pouch age during lactation. Error bars show Mean ± 1.0 SE.

When splitting the same dataset for bettong milk produced for foster young further into TAD in weeks (Fig.4.15), three significant differences were found in week 8 [$F_{(4, 2)}$=24.434, p=0.040], week 14 [$F_{(5, 4)}$=8.881, p=0.027] and week 15 [$F_{(5, 4)}$=22.547, p=0.005].

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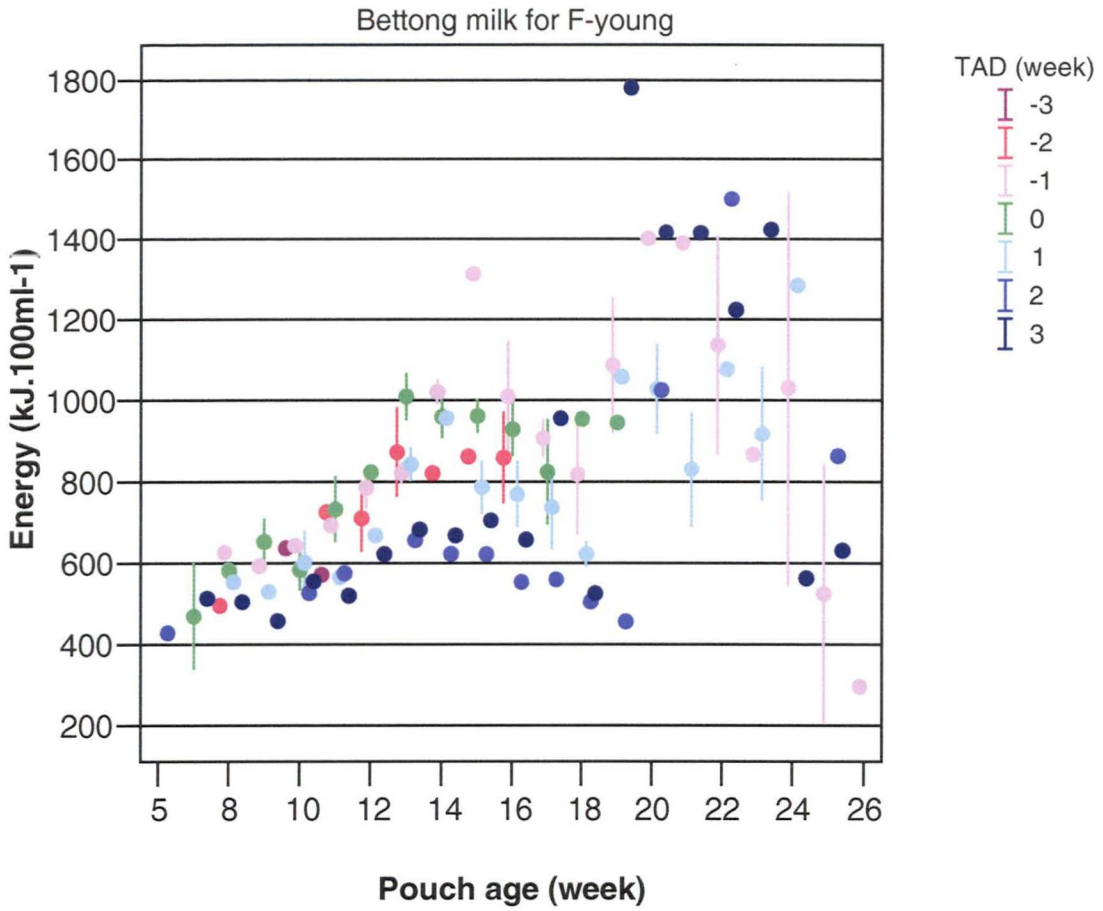

Fig.4.15: Changes in energy content (kJ.100ml^{-1}) of bettong milk produced for foster young (bettong) with a transfer age difference of –3 (dark purple, N=1/2), -2 (medium purple, N=2/10), -1 (light purple, N=2/32), 0 (green, N=4/31), 1 (light blue, N=2/29), 2 (medium blue, N=2/13) or 3 (dark blue, N=1/19) weeks compared to pouch age during lactation. Error bars show Mean ± 1.0 SE.

The data points for the energy content of the samples produced for same age and younger transfer offspring (TAD: 0 and –1 week) retained their relationship, referred to as 'layer-effect', to the older foster young (TAD: 2 and 3 weeks) with higher values between week 12 and 18. A summary of all significant differences between transfer and age groups for various milk components is provided in Table 4.1.

Table 4.1: Summary of all significant One Way ANOVA results (F-ratio/p-value) and *Post-hoc* comparison listed by milk component and pouch age week. 'Species' relates to the mother producing the milk, 'Transfer category' to the young drinking the milk. Abbreviations used in *post-hoc* comparisons for transfer category: CF=cross-foster, F=foster, orig=original, S=same, Y=younger, O=older, >=significant difference, comma=no significant difference, numbers equal TAD in weeks compared to pouch age (negative value=younger, positive value=older).

week	species	F-ratio	p-value	LSD results (Mean ± Standard error)
Transfer categories				
volume				
10	pot	$F_{(2, 5)}$=6.031	p=0.046	CF (0.37±0.03), F (0.40±0.00) > orig (0.15±0.08)
27	bet	$F_{(1, 4)}$=9.552	p=0.037	CF (1.40±0.10) > orig (0.53±0.18)
28	bet	$F_{(1, 2)}$=22.231	p=0.042	orig (1.15±0.15) > CF (0.30±0.10)
carbohydrate				
8	bet	$F_{(2, 8)}$=7.857	p=0.013	F (13.79±0.34) > CF (9.75±2.49), orig (10.15±1.02)
24	pot	$F_{(1, 1)}$=201.720	p=0.045	F (3.06±NA) > CF (2.13±0.04)
26	pot	$F_{(2, 1)}$=212.028	p=0.049	orig (4.57±NA) > CF (0.53±0.11)
lipid				
13	bet	$F_{(2, 14)}$=5.092	p=0.022	F (9.60±0.91) > orig (4.76±0.83)
16	pot	$F_{(2, 1)}$=28729.672	p=0.004	F (8.85±NA) > orig (7.82±NA) > CF (4.12±0.01)
17	pot	$F_{(2, 2)}$=104.012	p=0.010	F (12.03±NA) > CF (6.17±0.08), orig (4.44±0.43)
protein				
8	bet	$F_{(2, 8)}$=5.614	p=0.030	CF (10.27±1.97) > F (7.40±0.33), orig (6.43±0.07)
13	pot	$F_{(2, 4)}$=8.085	p=0.039	orig (7.92±0.22) > F (6.86±0.01)
16	pot	$F_{(2, 1)}$=288.000	p=0.042	F (10.69±NA) > CF (9.54±0.03)
26	bet	$F_{(2, 3)}$=11.250	p=0.040	orig (16.03±0.92), CF (13.99±1.21) > F (7.10±NA)
energy				
6	bet	$F_{(1, 1)}$=6383.530	p=0.008	CF (530.77±0.57) > orig (451.45±NA)
8	bet	$F_{(2, 8)}$=7.797	p=0.013	CF (605.04±35.13), F (561.82±17.80) > orig (434.80±26.26)
17	pot	$F_{(2, 2)}$=174.852	p=0.006	F (928.24±NA) > CF (680.24±13.10) > orig (590.13±6.94)
general transfer age difference - foster young				
volume				
7	bet	$F_{(1, 2)}$=25.000	p=0.038	S (0.50±0.00) > O (0.25±0.05)
carbohydrate				
15	bet	$F_{(2, 7)}$=4.950	p=0.046	S (12.11±1.57) > Y (7.57±0.77)
lipid				
9	bet	$F_{(2, 4)}$=9.614	p=0.030	S (5.11±0.32) > O (2. 86±0.30)
11	bet	$F_{(2, 8)}$=6.030	p=0.025	S (6.48±1.14), Y (5.94±0.29) > O (3.85±0.22)
protein				
15	bet	$F_{(2, 7)}$=5.459	p=0.037	Y (13.56±0.73) > O (10.33±0.62)

(Table 4.1 continued)

week	species	F-ratio	p-value	LSD results (Mean ± Standard error)
general transfer age difference - foster young				
total solids				
13	bet	$F_{(2, 7)}$=5.525	p=0.036	S (29.47±0.05) > O (23.51±1.58)
15	bet	$F_{(2, 7)}$=10.653	p=0.008	Y (33.20±3.26) > O (21.69±0.71), S (25.90±0.94)
energy				
15	bet	$F_{(2, 7)}$=6.832	p=0.023	Y (1163.45±150.50) > O (725.54±46.60)
general transfer age difference - cross-foster young				
carbohydrate				
10	pot	$F_{(1, 1)}$=77120.333	p=0.002	Y (12.71±0.01) > S (9.08± NA)
protein				
9	pot	$F_{(1, 1)}$=432.000	p=0.031	Y (6.53±0.03) > S (5.49± NA)
transfer age difference in weeks - foster young				
carbohydrate				
12	bet	F(5, 4)=13.048	p=0.014	0 (14.79±0.56), 3 (13.88±NA), 1 (13.69±0.26), -1 (13.53±1.49), -2 (12.88±0.05) > -3 (4.27±NA)
13	bet	F(5, 4)=16.435	p=0.009	1 (13.61±0.06) > 2 (12.21±NA), 0 (12.11±0.11), -1 (11.18±0.14), -2 (11.14±0.52) > 3 (9.79±NA)
lipid				
14	bet	F(5, 4)=6.432	p=0.048	[-1 (14.49±0.18), 1 (12.35±0.49), 0 (11.64±1.41) > 2 (4.30±NA)], [-1, 1 > 3 (6.32±NA)]
15	bet	F(5, 4)=8.055	p=0.033	-1 (22.79±1.55) > 0 (12.80±2.07), -2 (9.93±NA), 1 (8.85±0.47), 3 (8.18±NA), 2 (5.53±NA)
21	bet	F(2, 2)=19.638	p=0.048	3 (26.32±NA), -1 (24.66±0.32) > 1 (10.91±2.49)
protein				
14	bet	F(5, 4)=33.041	p=0.002	[1 (13.23±0.10), 0 (13.18±0.23) > -1 (11.89±0.06) > -2 (10.60±NA), 3 (10.06±NA)], [1, 0 > 2 (10.92±NA)
total solids				
15	bet	F(5, 4)=12.089	p=0.016	-1 (36.19±2.28) > -2 (27.23±NA), 0 (25.90±0.94), 1 (22.45±1.27), 3 (21.41±NA), 2 (20.45±NA)
energy				
8	bet	F(4, 2)=24.434	p=0.040	[-1(627.43±NA) > 1 (554.52±NA), 3 (505.45±NA), -2 (497.05±NA)], [0 (582.77±6.67) > 3 (505.45±NA), -2 (497.05±NA)]
14	bet	F(5, 4)=8.881	p=0.027	-1 (1021.53±27.81), 0 (960.42±49.78), 1 (957.14±3.36) > 3 (668.92±NA), 2 (623.95±NA)
transfer age difference in weeks - cross-foster young				
carbohydrate				
9	bet	F(1, 1)=1452.000	p=0.017	1 (12.69± NA) > 2 (11.69±0.02)
protein				
21	bet	F(2, 1)=369.188	p=0.037	2 (14.85±0.03) > 3 (14.05±NA), 1 (13.56±NA)

Most of the significant differences between the age groups indicated a disadvantage for older young by being provided with lower levels of the analysed milk components. These results suggest that mothers do not change the milk composition according to the needs of the young. However, significant differences should not be overrated due to the small number of animals per category.

4.4 Discussion

The milk analysis has shown that pouch young transfers did not essentially affect the milk composition or the timing of change of milk components. This applied to intra- and inter-species transfers as well as synchronicity of transfers. The application of techniques measuring the milk intake by suckling young, e.g. tritiated water turnover (MacFarlane *et al.* 1969) or ^{22}Na turnover (Green & Newgrain 1979), was avoided to minimize stress for the transferred young. However, the milk volume, collected from the mother following a three hours separation from the associated young, was measured and used as an estimate for potential milk intake over the period of isolation. Mean maximum volumes in Tasmanian bettong milk and Long-nosed potoroo milk produced for original, fostered and cross-fostered young were associated with the time of pouch vacation and the following three weeks (week 15 to 18 for bettong milk, week 18 to 21 for potoroo milk). Values subsequently decreased to near zero at time of weaning (week 26 to 34 for bettong milk, week 28 to 35 for potoroo milk). Rose *et al.* (2003) found a similar pattern in the milk volumes produced for free-living and captive Tasmanian bettongs, however, weaning occurred considerably earlier (week 22) in comparison to the study animals in this thesis.

The pattern of increased carbohydrate content in early milk and rapid decrease in mid-lactation following pouch vacation is present in both bettong and potoroo milk. The described pattern was less obvious in other studies conducted on the same species (Crowley *et al.* 1988, Smolenski & Rose 1988, Rose *et al.* 2003). While Crowley *et al.* (1988) and Smolenski and Rose (1988) described a similar range of carbohydrate values for Long-nosed potoroos, the latter authors also found a lower carbohydrate content in Tasmanian bettong milk for early and mid-lactation (majority of values between 8 and 12g.100ml^{-1} compared to 11

and 14g.100ml^{-1} in the present study in weeks 5 to 15). On the contrary, results for carbohydrate content found by Rose *et al.* (2003) were slightly elevated for the same period in comparison (most values are near 15g.100ml^{-1} or above for captive bettongs).

The findings for lipid content in Long-nosed potoroo milk in early and mid-lactation correspond well with the results shown by Smolenski and Rose (1988). Crowley *et al.* (1988) found generally lower values for the same period. The mentioned authors do not show the subsequent decrease in lipid concentration towards weaning. This might be due to the fact that data was only displayed up to week 25 of lactation or weaning might have been defined differently. The described lipid concentrations for Tasmanian bettongs in this study corresponded well with the findings of Rose *et al.* (2003), but were slightly higher in early and mid-lactation compared to the results of Smolenski and Rose (1988) with values ranging from close to zero up to ca. 7g.100ml^{-1} from week 5 to 15 compared to ca. 3 to 12g.100ml^{-1} for the same period in the present study. Again, the data of the research mentioned above does not describe the final decrease in lipid content towards weaning. Data in those previous studies was only displayed up to week 20 and week 24 respectively.

The simultaneous decrease in carbohydrate content and increase in lipid concentration in milk of both species at time of pouch vacation applies to marsupials in general and suggests a higher energy demand of the young for independent thermoregulation and increased muscular activity associated with locomotion and skeleto-muscular support once it left the pouch permanently or is deposited in a nest (Green & Merchant 1988).

Protein concentrations in both bettong and potoroo milk corresponded well with the results shown by Smolenski and Rose (1988) and Rose *et al.* (2003). Crowley *et al.* (1988) found slightly lower protein concentrations in potoroo milk in mid-lactation (ca. 5 opposed to ca. 7g.100ml^{-1}). The initial peak in protein concentration of bettong milk at time of pouch vacation was also produced in

the data presented by Smolenski and Rose (1988); however, the relevance of this pattern is not understood yet.

The total solid fraction in the bettong milk was lower throughout most of lactation compared to other studies. Rose *et al.* (2003) found a total solid content of ca. 27 to 33% in early and mid lactation opposed to 18 to 24% in the present study. The results of Smolenski and Rose (1988) were only slightly elevated (varying from 20 to 30% between week 5 and 20 of lactation), but increased greatly towards the end of lactation. This trend was found by other authors as well (Crowley *et al.* 1988, Rose *et al.* 2003), but was only present in the analysed total solid fraction of potoroo milk in this study. The over all results for total solids in potoroo milk corresponded well with the findings of Crowley *et al.* (1988), but were lower up to week 20 of lactation compared to the results of Smolenski and Rose (1988). The described variations in milk composition in the above studies might be related to differences in nutrition (Rose *et al.* 2003).

The significant differences for certain milk components in regards to transfer category, transfer age and TAD were isolated findings in both species. This suggests that mothers did not change the milk composition according to the needs of the transferred young. The results of this study supported findings of Trott *et al.* (2003) that the milk composition remained essentially unchanged regardless of the transferred young's age.

Merchant and Sharman (1966) placed an additional young into the pouch of a red kangaroo foster mother without removing the original young. Both young suckled from the same teat and were reared successfully, which suggested an increase in milk quantity since no retarded growth was reported for either young. Their study also showed that the continued milk demand by the transferred young (appropriate for its species) led to an extended lactation period in the recipient mother, which indicates that milk quantity might be adjustable 'on request'. However, growth data of an older bettong transferee (TAD: three weeks) in the current study showed that a lack in milk quality could not be compensated with possibly increased milk quantity.

Chapter 5: Growth and Development

5.1 Introduction

Kirkwood and Mace (1996) pointed out the importance of reproductive rate enhancing techniques combined with an understanding of normal growth patterns for the management of captive breeding programs. This should insure that reared young become successful breeders once matured. They also noted that growth (increase in size) and development (change in morphology, anatomy, chemical composition and function of the organism) are often not closely correlated across species. Therefore information from one species will not necessarily predict the timing of key developmental stages in another.

Various aspects of growth and development have been investigated in the past, e.g. the connection between growth and metabolism (von Bertalanffy 1957), evolution and adaptive significance of postnatal growth rates (Case 1978), growth rate and calorie intake from the milk (Bernhart 1961), growth in captive and wild animals (Taylor & Rose 1987, Delaney & De'ath 1990), growth and reproduction (Poole *et al.* 1985, Bryant 1989) and to a great extent between growth and age estimation of the young (for example Shield & Woolley 1960, Maynes 1972, Rose & McCartney 1982, Close & Bell 1990).

Kirkwood and Mace (1996) pointed out that milk production of the mother is designed to meet the demands of the young for 'normal' growth. They also stated that undernutrition in growing young can result in reduced adult size, while excess food intake causes ingestion of excess energy as well as deposition of abnormal amounts of fat.

Most neural development in marsupial young occurs after birth while dependant on milk with the immature brain being sensitive to dietary changes (Nelson 1988). The transfer age difference (TAD) in young for potential transfers has to be carefully considered to avoid disturbance in brain growth due to nutritional deficiencies.

Since the growth rate of the young is regulated by the milk production rate and changing milk composition (Green 1984) irrespective of the age of the young (Trott *et al.* 2003), the TAD in asynchronous intra-species transfers and inter-species pouch young transfers has to be restricted. Clark (1968) found mostly normal growth rates for young with a TADs of less than five days as well as between 5 and 20 days, but reported a higher death rate combined with the occurrences of obesity as well as retarded growth for young with a TAD greater than 20 days. Merchant and Sharman (1966) found mostly normal growth rates for the young transferred at an age of less than one day and between 2 and 25 days, with the exception of one animal (*W. bicolor* young transferred to *M. rufa* transfer mother) in the latter category, which showed accelerated growth and early sexual maturity. They reported retarded growth in 3 out of 12 young transferred at an age greater than 40 days and mostly normal growth in the remaining young (4 deaths).

The measurement of growth and development provides knowledge of basic biology as well as individual progress of captive animals, and can reveal fluctuations, which can be used as an early indicator for disease or suboptimal management (Kirkwood & Mace 1996).

The aim of this study was to investigate the impact of pouch young transfer and in particular of transfer age difference on growth and development of the transferred young on a more detailed level (defined in weeks).

5.2 Methods

When the mothers were being milked, measurements of the young's foot-, tail- and head-length, testis size as well as body mass were obtained. Each measurement and calculations based upon it are presented below. Although milking was not conducted until the pouch young reached a minimum age of five weeks, measurements of growth and development data of the young were collected on a weekly basis from the earliest possible age (measured in the pouch). An exception was made for extremely stressed mothers (usually potoroos) in which case measuring and/or milking was only performed each fort-

night. Measurements were obtained throughout pouch life until weaning and thereafter depending on the stress of the involved animals. Body mass was monitored throughout the whole period animals were kept in captivity as part of colony management.

5.2.1 Body measurements and associated calculations

The body mass of pouch young up to small sub-adults was determined by placing the individual in a cotton bag on an electronic balance (Mettler PJ3600 DeltaRange®, Switzerland). The measurement was obtained to the nearest 0.01g. Larger animals in hessian bags were placed on a spring balance (Salter, No.235, to weigh 5 kg by 25 g).

Growth rates change throughout the animal's life and have been described for the Tasmanian bettong (Rose 1984, 1989*b*) and the Long-nosed potoroo (Bryant 1989). The instantaneous relative growth rate (k) (Maynes 1976) was chosen to detect possible differences between the transfer groups (cross-foster, foster and original bettongs and potoroos respectively), when relative growth occurs at a constant rate. It was calculated using the following equation:

$$k = \frac{\log_e \text{weight}_2(g) - \log_e \text{weight}_1(g)}{\text{day}_2 - \text{day}_1}$$

Various measurements have been taken with vernier callipers (Mitutoyo, Japan) to the nearest 0.1mm. The head length was measured from the tip of the rhinarium of the young to the back of its skull. The foot length was taken from the back of the heel to the tip of the toe without including the toenail. Soil caught under toenails from young capable of leaving the pouch had to be removed prior to measuring. The tail length was measured from the rump to the tip of the tail. If the tail length exceeded the measurable range of the callipers, a metal tape measure (Eagle, China) was used instead.

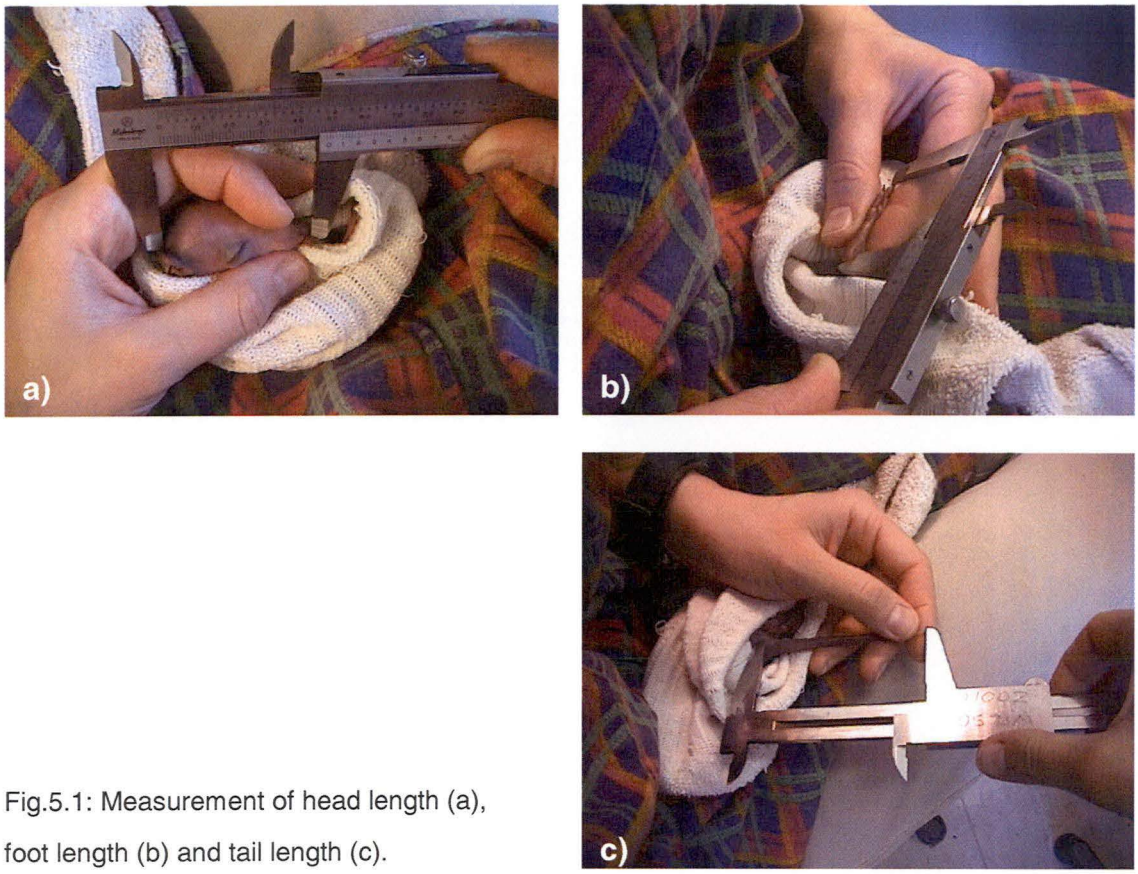

Fig.5.1: Measurement of head length (a), foot length (b) and tail length (c).

An index of body condition was calculated for each individual young as ratio of body mass (g) / head length (mm). Testes measurements were collected to determine if an age related difference existed in reaching sexual maturity depending on the transfer category and/or age difference. Length (L), breadth (b_1) and width (b_2) of both testes were measured for male study animals. The formula for an ellipsoid was used to calculate the testicular volumes as outlined in Rose *et al.* (1997):

$$\frac{4}{3} \times \pi \times \frac{L}{2} \times \frac{b_1}{2} \times \frac{b_2}{2}$$

Combined testes weights were derived from the calculated volume after Harcourt *et al.* (1995):

weight (g) = 2 x volume of a single testis (cm^3) x 1.1

All body measurements (except body mass) were taken as duplicates and the calculated average was used for the subsequent analysis. Only two young were hand-reared as part of this study. Additional hand-rear data was obtained for nine Tasmanian bettongs from the Tasmanian Carers Network (registered wildlife carers, Department of Primary Industries, Water and Environment [DPIWE]).

5.2.2. Development assessment

The appearance and development of certain body features and their use (Table 5.1) was assessed for both bettongs and potoroos (Fig.5.2) as part of the measuring process.

Table 5.1: Selection of developmental stages for certain body characteristics. Modified from Rose (1984)

body feature	**development stage**
eyes	1) closed, 2) open
external ears	1) pointing forward, 2) free, 3) pointing backwards, 4) erect
mouth	1) anterior end open only, 2) lateral lip groove develops, 3) open laterally, 4) fully open
sucking	1) young firmly attached to teat, 2) able to release teat, 3) suckling from outside
pigment	1) absent, 2) nose, ears, claws, 3) extremities, 4) back, 5) body
vibrissae	1) absent, 2) papillae evident, 3) all present, 4) fully developed
hair	1) absent, 2) fine hair, 3) guard hair erupting, 4) fully furred
vocalisation	1) mute, 2) gentle calls, 3) squeaking, 4) hissing
locomotion	1) none, 2) crawling, 3) slow upright movement, 4) hopping
pouch life	1) continuously in the pouch, 2) in and out, 3) out permanently

The developmental stage for all body characteristics except locomotion could be determined without removing the young from the pouch. Locomotive abilities were only examined when the young was routinely removed from the teat for subsequent milk collection from the mother (Fig.5.2).

Fig.5.2: Potoroo pouch young begins to move slowly in an upright position. Its eyes are closed, ears point backwards, the mouth is fully open and it is therefore able to release the teat. Pigmentation is present on the whole body; vibrissae and fine hair are present. The young squeaks when placed onto the foam.

5.3 Results

Body measurements were obtained for a total of 110 Tasmanian bettongs and 46 Long-nosed potoroos. These measurements are presented for both species together to facilitate cross-species comparison for the different transfer categories and TADs. Body measurements are only shown for the first year in the life of the study animals. It is indicated in the text, if described trends changed further in time. All data (unless otherwise indicated) are presented as mean (M) ± standard error (SE). In the graph description 'N' is provided as the number of individuals/datapoints.

5.3.1 Body measurements for each transfer category

5.3.1.1 Body mass

The results for mean body mass described a sigmoid trend for all four transfer categories. There was a steady increase from week 1 (orig: 1.29g ± 0.11) to week 10 (CF: 47.94g ± 8.23, orig: 58.32g ± 3.34, F: 70.71g ± 8.62), which was followed by a more rapid increase until week 30 (CF: M=1202.00g, orig: 1499.80g ± 28.78, F: 1540.00g ± 30.00, HR: M=1550.30g). Results for cross-foster young fell behind during the rapid increase and reached equivalently high

values 12 weeks later (week 42: M=1558.80g). Results for all young subsequently formed a plateau after having reached an adult body mass of ca. 1700g (Fig.5.3.1).

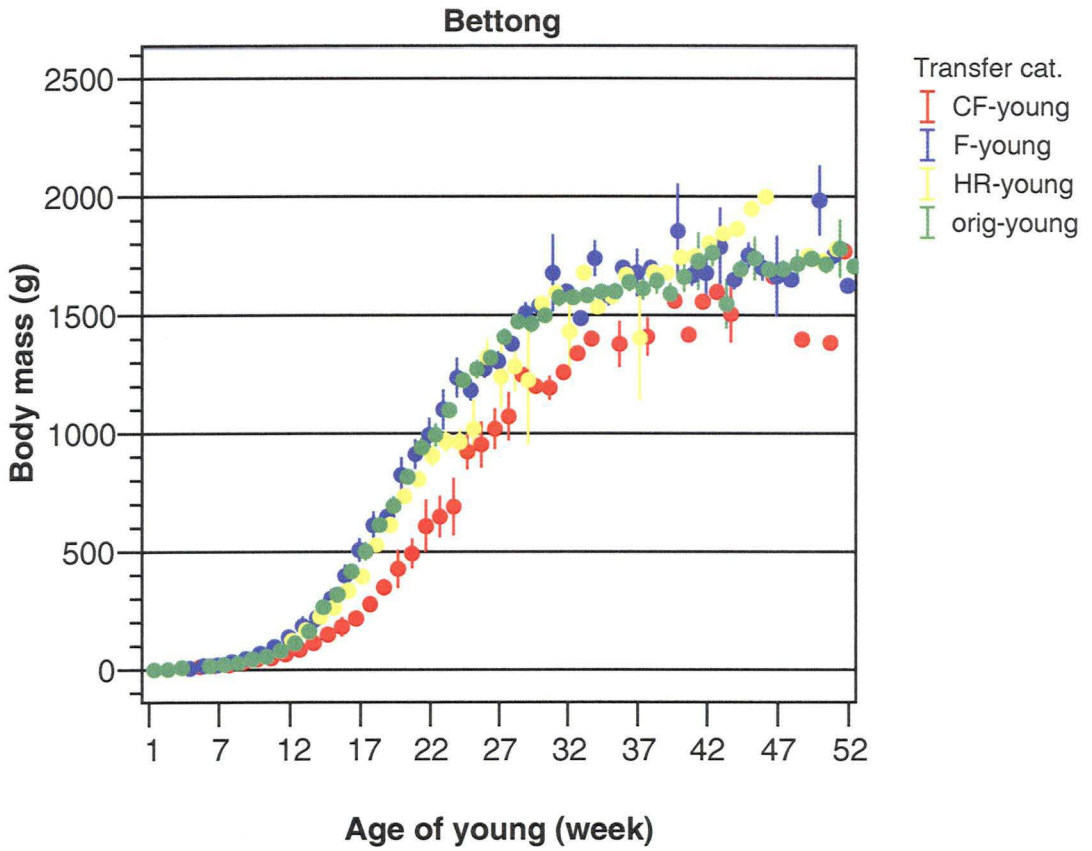

Fig.5.3.1: Changes in body mass (g) of cross-fostered (red, N=5/80), fostered (blue, N=14/197), hand-reared (yellow, N=10/265) and original (green, N=59/424) bettong young up to the age of 52 weeks. Error bars show Mean ± 1.0 SE.

The body mass of cross-foster bettongs increased less rapidly compared to the other transfer categories, resulting in significant differences in mean body mass between the groups throughout lactation and in early sub-adulthood (Table 5.2).

Table 5.2: Significant results of the One way Anova test (F-ratio and p-value) for differences in body mass (g) between the bettong transfer groups listed by weeks of age accompanied by group comparison results (LSD test); (CF=cross-foster, F=foster, HR=hand-reared, orig= original, >=significant difference, comma=no significant difference).

age	F-ratio	p-value	LSD results (Mean ± Standard error)
8	$F_{(2, 10)}$=4.677	p=0.037	F (35.95±2.59) > CF (22.75±4.57)
9	$F_{(2, 14)}$=5.425	p=0.018	F (49.69±4.50), orig (46.82±1.95) > CF (29.23±5.47)
14	$F_{(3, 39)}$=3.304	p=0.030	orig (266.46±16.12), HR (226.51±11.67), F (220.68±33.63) > CF (116.59±28.92)
15	$F_{(3, 42)}$=3.206	p=0.033	orig (319.36±23.21), F (301.91±35.16), HR (266.29±18.35) > CF (151.93±19.04)
16	$F_{(3, 43)}$=3.562	p=0.022	[orig (417.40±25.25) > HR (336.82±24.39), CF (183.94±35.29)]; [F (398.53±43.45) > CF]
17	$F_{(3, 44)}$=5.531	p=0.003	[F (508.03±44.91), orig (502.30±34.19), HR (396.54±26.60) > CF (219.32±24.65)]; [F, orig > HR]
18	$F_{(3, 39)}$=6.936	p=0.001	orig (614.33±16.11), F (613.26±52.36), HR (529.08±26.16) > CF (279.28±26.24)
19	$F_{(3, 34)}$=6.069	p=0.002	orig (694.84±34.23), F (649.05±40.83), HR (615.09±28.87) > CF (350.08±29.27)
20	$F_{(3, 39)}$=7.595	p<0.001	[F (825.66±69.18), orig (817.55±28.23), HR (736.73±23.33) > CF (428.79±75.32)]; [orig > HR]
21	$F_{(3, 33)}$=13.926	p<0.001	[orig (941.84±36.73), F (914.15±58.15), HR (808.00±19.12) > CF (493.63±57.40)]; [O > HR]
22	$F_{(3, 22)}$=5.007	p=0.009	orig (996.15±43.52), F (995.30±67.67), HR (907.50±38.39) > CF (609.90±105.50)
23	$F_{(3, 27)}$=12.247	p<0.001	[F (1103.29±78.26), orig (1100.62±26.80), HR (969.23±37.79) > CF (649.97±82.95)]; [orig > HR]
24	$F_{(3, 30)}$=15.746	p<0.001	[F (1237.04±78.74), orig (1226.49±23.22), HR (966.08±49.09) > CF (692.23±115.91)]; [F, O > HR]
25	$F_{(3, 20)}$=4.757	p=0.012	orig (1273.49±33.85) > HR (1021.83±118.42), CF (924.30±69.24)
26	$F_{(3, 17)}$=7.771	p=0.002	HR (1324.65±69.65), orig (1321.40±27.82), F (1274.93±32.66) > CF (955.55±92.25)
27	$F_{(3, 16)}$=8.603	p=0.001	[orig (1408.20±28.65), F (1306.85±35.47), HR (1242.53±125.93) > CF (1021.73±80.02)]; [orig > HR]
28	$F_{(3, 14)}$=5.158	p=0.013	orig (1473.10±28.05) > HR (1285.32±99.33), CF (1074.40±96.60)
30	$F_{(3, 11)}$=3.655	p=0.048	HR (1550.30±NA), F (1540.00±30.00), orig (1499.80±28.78) > CF (1202.00±NA)
31	$F_{(3, 14)}$=4.995	p=0.015	F (1678.33±156.96), HR (1596.00±NA), orig (1575.53±32.46) > CF (1194.85±45.85)
33	$F_{(3, 19)}$=4.195	p=0.019	HR (1680.00±NA), orig (1584.61±23.66) > CF (1340.70±13.30)
34	$F_{(3, 17)}$=3.943	p=0.026	F (1740.00±70.00), orig (1599.76±25.96) > CF (1402.20±22.80)
46	$F_{(2, 11)}$=8.556	p=0.006	HR (2000.00±NA) > F (1700.00±48.31), orig (1692.97±19.57)
49	$F_{(2, 4)}$=87.023	p=0.001	HR (1750.00±NA), orig (1738.00±10.68) > CF (1400.00±NA)

Results for potoroo young followed the same trend described for bettong, but instead of a plateau phase the rapid increase was followed by another steady increase (Fig.5.3.2). In the first 13 weeks of pouch life, potoroo young gained weight steadily with values rising from less than 1g in week 1 (orig: M=0.74g) to just under 100g in week 13 for cross-foster young (CF: 96.68g ± 19.21, orig: 74.64g ± 15.20, F: 63.72g ± 7.66). Values increased more rapidly up to week 34 (CF: 791.17g ± 20.05, F: M=834.00g, orig: 870.26g ± 12.27, HR: M= 935.00g). Subsequently the body mass kept rising for most young until they reached two years of age (females: ca. 1200g, males: ca. 1500g). Hand-reared young continued the rapid increase in body mass slightly longer, reaching 1200g in week 51 well before the young of the other categories (HR: M=1231.20g, orig: M=1015.00g, CF: 960.15g ± 20.15). A significant difference was found between the groups in week 34 [$F_{(3, 6)}$=7.344, p=0.020]. The *post-hoc* comparisons are summarised in Table 5.3.

Table 5.3: Significant results of the One way Anova test (F-ratio and p-value) for differences in body mass (g) between the potoroo transfer groups listed by weeks of age accompanied by group comparison results (LSD test); (CF=cross-foster, F=foster, HR=hand-reared, orig= original, >=significant difference, comma=no significant difference).

age	F-ratio	p-value	LSD results (Mean ± Standard error)
20	$F_{(3, 17)}$=3.539	p=0.037	CF (438.70±44.32) > orig (365.35±6.05), HR (347.52±5.92)
21	$F_{(3, 10)}$=4.767	p=0.026	CF (525.61±38.49) > orig (428.28±3.48), HR (393.38±5.28)
34	$F_{(3, 6)}$=7.344	p=0.020	HR (935.00±NA), orig (870.26±12.27) > CF (791.17±20.05)

A 'layer-effect' (data points for displayed categories retain their relationship) was found in mid and late lactation with cross-foster potoroos reaching higher body masses than foster-potoroos while hand-reared and original young remained mostly at equal levels. This resulted in significant differences in mean body mass between the potoroo transfer groups in week 20 [$F_{(3, 17)}$=3.539, p=0.037] and week 21 [$F_{(3, 10)}$=4.767, p=0.026]. There was greater fluctuation in the results after weaning, which resulted in the blending of most layers.

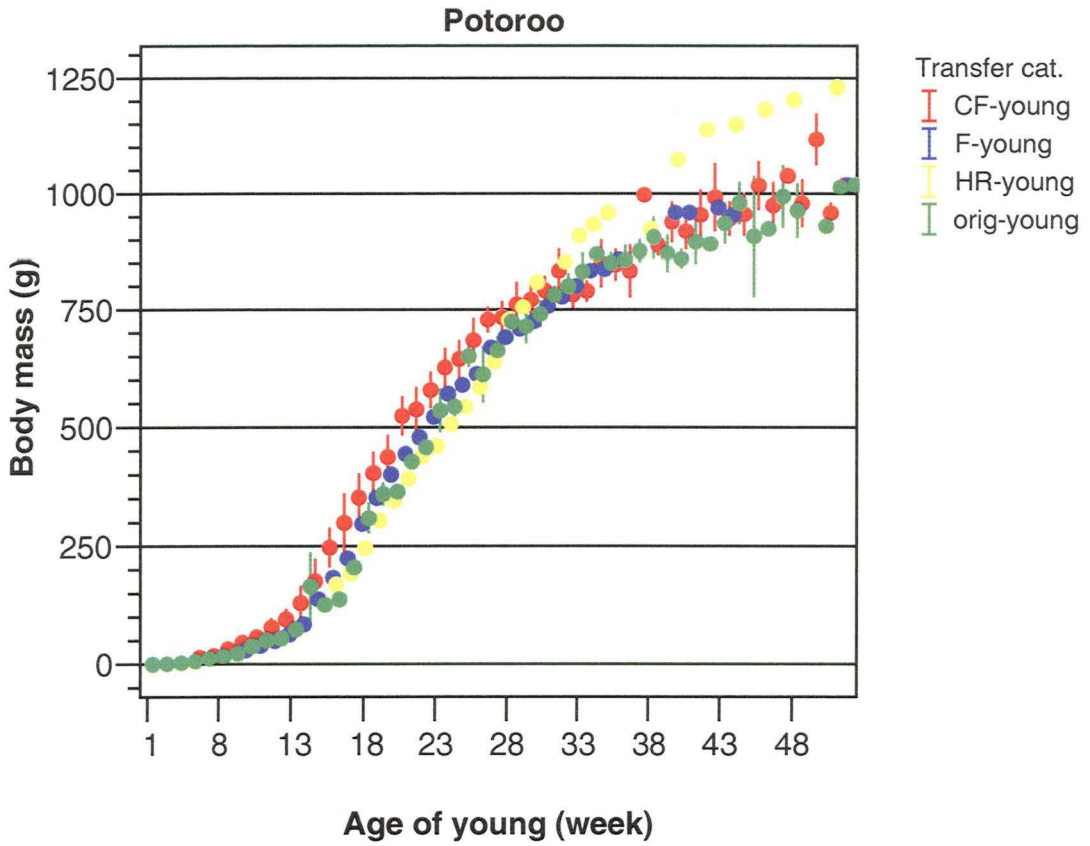

Fig.5.3.2: Changes in body mass (g) of cross-fostered (red, N=7/172), fostered (blue, N=2/37), hand-reared (yellow, N=1/60) and original (green, N=24/163) potoroo young up to the age of 52 weeks. Error bars show Mean ± 1.0 SE.

Five periods were identified in the first year of the bettong's life when growth (based on body mass measurements) occurred at a constant rate. These periods appeared to be related to changes in the young's development. The mean instantaneous relative growth rate (k) calculated for each period is inversely related to the age of the young. The results in Table 5.4 support the above finding for bettong body mass. Cross-foster young had the lowest k-value in the second growth period (age: 7 - 15 weeks) compared to the other transfer categories, which resulted in body mass values falling behind. The cross-foster k-values were comparable with the results of most other transfer categories (age: 16 - 21 weeks) or even higher in the following growth periods (age: 22 - 52 weeks); however, it took the young 12 additional weeks to reach equivalently high body mass values compared to the other transfer categories (Fig.5.3.1).

Statistical analysis for age periods, which provided sufficient data, showed no significant difference in mean instantaneous growth rate between the transfer categories for both bettong and potoroo data.

Table 5.4: Mean instantaneous relative growth rate (k) for the bettong transfer categories during periods of constant growth accompanied by the age and developmental stage of young. Empty cells indicate unavailability of data.

transfer cat.	age (weeks)	k (LNg.day^{-1})	Stage of development
original	1 - 4	8.72 x 10-2	early pouch life, young is firmly attached to the teat
foster			
cross-foster			
hand-reared			
original	7 - 15	4.66 x 10-2	mid to late pouch life until PEP, young is able to release the teat
foster		5.10 x 10-2	
cross-foster		3.57 x 10-2	
hand-reared		3.60 x 10-2	
original	16 - 21	2.71 x 10-2	YAF, still sucking
foster		2.75 x 10-2	
cross-foster		2.73 x 10-2	
hand-reared		3.26 x 10-2	
original	22 - 32	7.04 x 10-3	weaning to subadult
foster		9.19 x 10-3	
cross-foster		1.13 x 10-2	
hand-reared		7.77 x 10-3	
original	32 - 52	8.43 x 10-4	subadult to adulthood
foster		1.02 x 10-3	
cross-foster		1.34 x 10-3	
hand-reared		7.32 x 10-4	

Five periods of constant growth were also identified for the potoroo transfer categories (Table 5.5). They differed in age due to a longer pouch life and associated developmental changes. The mean instantaneous growth rates for the potoroo young were generally lower compared to bettong young with the exception of results for cross-foster young (age: 8 - 18 weeks) and for original and hand-reared young (age: 40 - 52 weeks). The slight growth advantage seen for cross-foster potoroo young (Fig.5.3.2) was mirrored in the k-results for growth period two (age: 8 - 18 weeks). The hand-reared potoroo young had the lowest k-result for growth period two, but the instantaneous growth rate subsequently (age: 18 - 52 weeks) increased in comparison with the other transfer categories.

Table 5.5: Mean instantaneous relative growth rate (k) for the potoroo transfer categories during periods of constant growth accompanied by the age and developmental stage of young. Empty cells indicate unavailability of data.

transfer cat.	age (weeks)	k (LNg.day^{-1})	Stage of development
original	1 - 7	7.75 x 10-2	early to mid-pouch life, young is attached to the teat
foster			
cross-foster			
hand-reared			
original	8 - 18	3.75 x 10-2	mid to late pouch life until PEP, young is able to release the teat
foster		4.12 x 10-2	
cross-foster		4.25 x 10-2	
hand-reared		2.51 x 10-2	
original	18 - 25	1.57 x 10-2	YAF, still sucking
foster		1.37 x 10-2	
cross-foster		1.43 x 10-2	
hand-reared		1.71 x 10-2	
original	27 - 38	2.54 x 10-3	weaning to subadult
foster		4.07 x 10-3	
cross-foster		2.86 x 10-3	
hand-reared		4.85 x 10-3	
original	40 - 52	1.28 x 10-3	subadult to adulthood
foster		6.97 x 10-4	
cross-foster		9.30 x 10-4	
hand-reared		1.76 x 10-3	

5.3.1.2 Head length

Measurements for head length in bettong young increased from birth to late lactation (week 22) at a mainly constant rate for most transfer categories (Fig.5.3.3). The mean head length at birth was less than 1cm (orig: 0.76cm ± 0.02) and increased to ca. 8cm in the following 22 weeks (F: 8.11cm ± 0.26, orig: 7.62cm ± 0.16, CF: 7.29cm ± 0.17, HR for week 23: M=7.90cm). Values for all categories rose to ca. 9cm (adult head length) by approximately week 40. Results showed a greater degree of fluctuation following the weaning process (week 28 onwards).

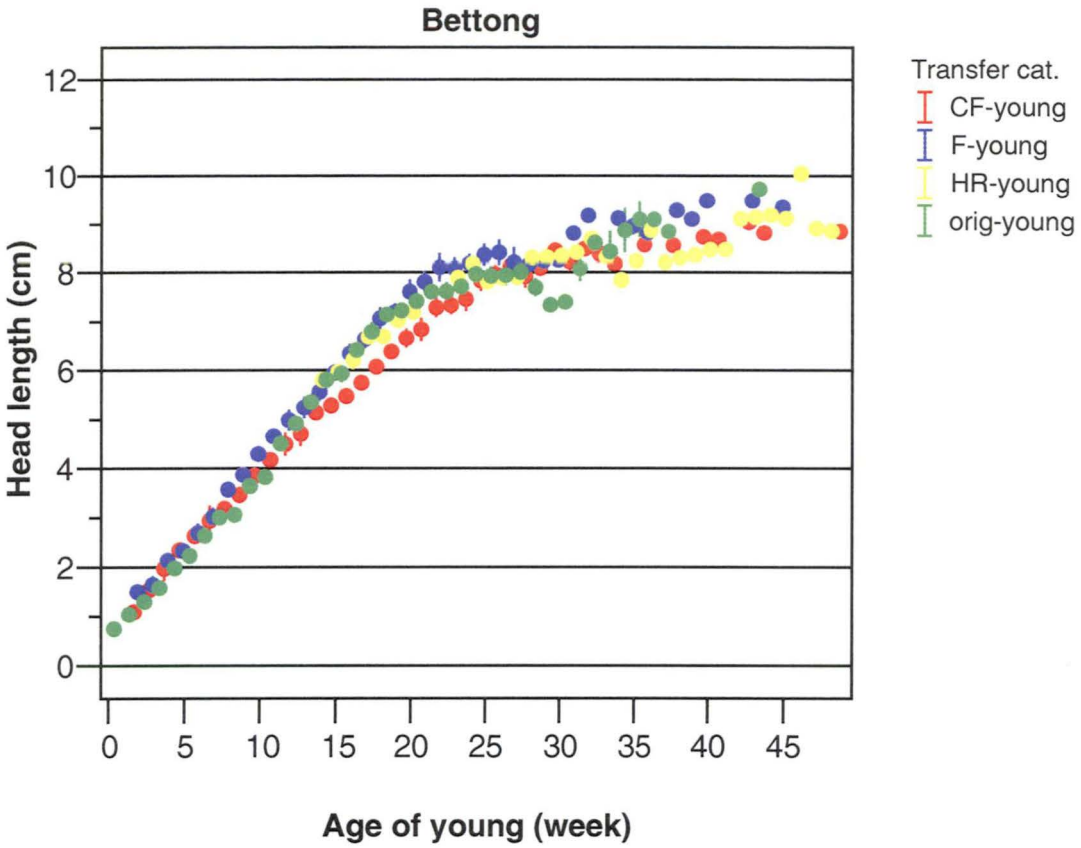

Fig.5.3.3: Changes in head length (cm) of cross-fostered (red, N=5/86), fostered (blue, N= 14/166), hand-reared (yellow, N=1/46) and original (green, N=71/234) bettong young up to the age of 52 weeks. Error bars show Mean ± 1.0 SE.

Results for mean head length in cross-foster bettongs fell behind between the weeks 14 and 24, which led to significant differences between the transfer groups (Table 5.6). Cross-foster young reached a mean head length of ca. 8cm about five weeks later than the foster bettongs.

Table 5.6: Significant results of the One way Anova test (F-ratio and p-value) for differences in head length (cm) between the transfer groups of both species listed by weeks of age accompanied by group comparison results (LSD test); (CF=cross-foster, F=foster, HR=hand-reared, orig=original, >=significant difference, comma=no significant difference).

age	F-ratio	p-value	LSD results (Mean ± Standard error)
bettong			
2	$F_{(2, 4)}$=11.332	p=0.023	F (1.51±NA) > orig (1.30±0.03) > CF (1.11±NA)
8	$F_{(2, 13)}$=8.385	p=0.005	F (3.58±0.09) > CF (3.19±0.12), orig (3.07±0.09)
14	$F_{(3, 21)}$=3.370	p=0.038	HR (5.81±0.04), orig (5.81±0.08) > CF (5.14±0.11)
17	$F_{(3, 19)}$=4.453	p=0.016	orig (6.80±0.17), HR (6.69±0.15), F (6.65±0.16) > CF (5.75±0.07)
18	$F_{(3, 15)}$=7.362	p=0.003	orig (7.15±0.04), F (7.07±0.20) > CF (6.08±0.08)
19	$F_{(3, 12)}$=4.871	p=0.019	orig (7.23±0.14), F (7.19±0.16) > CF (6.39±0.05)
21	$F_{(2, 11)}$=6.075	p=0.017	F (7.82±0.20), orig (7.61±0.13) > CF (6.84±0.21)
potoroo			
7	$F_{(1, 4)}$=8.455	p=0.044	CF (2.97±0.03) > orig (2.66±0.15)
9	$F_{(1, 8)}$=6.380	p=0.035	CF (3.67±0.13) > orig (3.41±0.04)

Potoroo measurements for mean head length (Fig.5.3.4) followed the above described trend for bettongs. Values increased from less than 1cm in week 1 (orig: 0.86cm ± 0.04) at a mostly constant rate to just under 8cm by week 22 (CF: 7.81cm ± 0.28, HR: M=7.70cm, orig: 7.64cm ± 0.10, F: M=7.10cm). Subsequently measurements increased more steadily with time with only cross-foster and hand-reared potoroos reaching an adult head length of ca. 10cm before the end of the first year (week 42).

Although cross-foster potoroos appeared to have a growth advantage from early to mid-lactation (week 7 to 16), significant differences in head length between the transfer groups were only found in week 7 [$F_{(1, 4)}$=8.455, p=0.044] and week 9 [$F_{(1, 8)}$=6.380, p=0.035]. *Post-hoc* comparisons are summarised in Table 5.6.

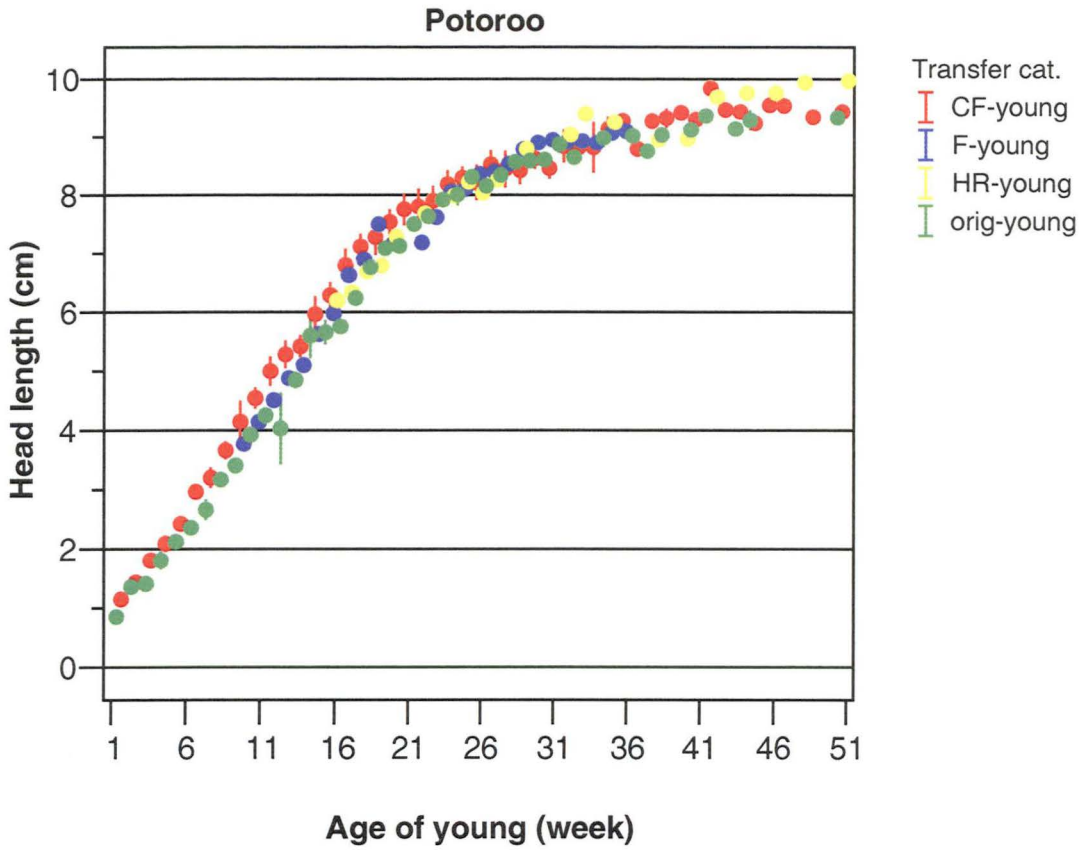

Fig.5.3.4: Changes in head length (cm) of cross-fostered (red, N=7/155), fostered (blue, N= 2/31), hand-reared (yellow, N=1/21) and original (green, N=31/123) potoroo young up to the age of 52 weeks. Error bars show Mean ± 1.0 SE.

5.3.1.3 Foot length

The increase in mean foot length occurred in three steps (Fig.5.3.5). Initially results from all categories rose at a similar rate from less than half a centimeter (orig: 0.46cm ± 0.02) to ca. 3cm in the first eight weeks of pouch life (F: 3.50cm ± 0.12, CF: 2.85cm ± 0.22, orig: 2.81cm ± 0.12, no values for hand-reared young available). The second increase in growth was even more rapid for original, foster and hand-reared bettongs gaining over 6cm in the following seven weeks leading up to pouch vacation (week 15, orig: 9.59cm ± 0.25, HR: 9.53cm ± 0.14, F: 9.52cm ± 0.25). Results for cross-foster bettongs fell behind in step two and reached equivalently high levels for mean foot length five weeks later (week 20: 9.64cm ± 0.17).

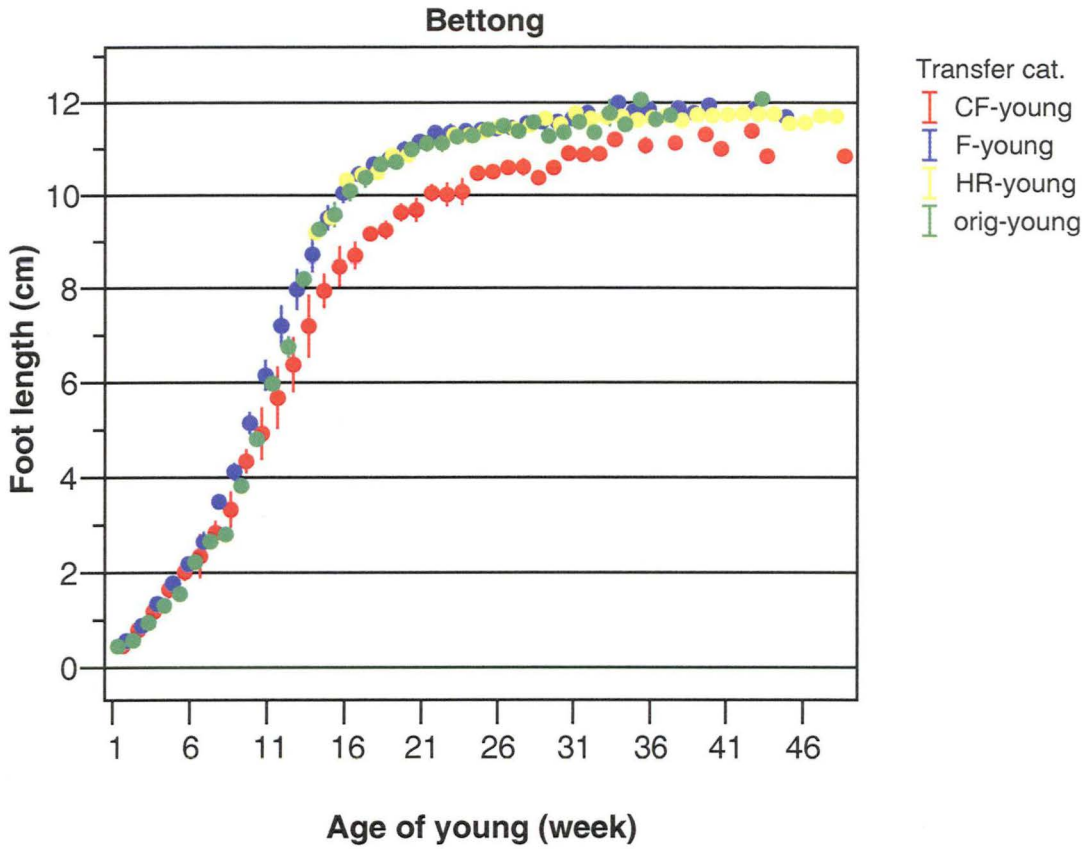

Fig.5.3.5: Changes in foot length (cm) of cross-fostered (red, N=5/86), fostered (blue, N= 14/166), hand-reared (yellow, N=2/41) and original (green, N=71/222) bettong young up to the age of 52 weeks. Error bars show Mean ± 1.0 SE.

In the final step, values for all categories resumed a steady increase with a subsequent plateau phase (week 30 onwards) after reaching the mean adult foot length level (ca. 12cm). Results for cross-foster bettongs followed the same trend, but remained lower (ca. 11cm) in the final plateau phase. Significant differences in mean foot length were detected between the transfer groups throughout lactation and early sub-adulthood (Table 5.7).

Table 5.7: Significant results of the One way Anova test (F-ratio and p-value) for differences in foot length (cm) between the transfer groups of both species listed by weeks of age accompanied by group comparison results (LSD test); (CF=cross-foster, F=foster, HR=hand-reared, orig=original, >=significant difference, comma=no significant difference).

age	F-ratio	p-value	LSD results (Mean ± Standard error)
bettong			
8	$F_{(2, 13)}$=8.064	p=0.005	F (3.50±0.12) > CF (2.85±0.22), orig (2.81±0.12)
9	$F_{(2, 18)}$=4.048	p=0.035	F (4.13±0.16) > CF (3.33±0.35)
14	$F_{(3, 19)}$=5.627	p=0.006	orig (9.27±0.07), HR (9.21±0.06), F (8.74±0.36) > CF (7.21±0.64)
15	$F_{(3, 26)}$=4.050	p=0.017	orig (9.59±0.25), HR (9.53±0.14), F (9.52±0.25) > CF (7.95±0.34)
16	$F_{(3, 21)}$=3.533	p=0.032	HR (10.34±NA), orig (10.11±0.21), F (10.06±0.20) > CF (8.47±0.41)
17	$F_{(3, 17)}$=10.046	p<0.001	F (10.47±0.14), HR (10.46±NA), orig (10.39±0.19) > CF (8.72±0.27)
18	$F_{(3, 15)}$=20.924	p<0.001	F (10.68±0.14), orig (10.68±0.09), HR (10.51±NA) > CF (9.18±0.13)
19	$F_{(3, 12)}$=23.314	p<0.001	HR (10.86±NA), F (10.79±0.10), orig (10.73±0.12) > CF (9.26±0.17)
20	$F_{(3, 14)}$=7.625	p=0.003	orig (11.00±0.14), F (11.00±0.15), HR (10.88±0.06) > CF (9.64±0.17)
21	$F_{(2, 11)}$=23.143	p<0.001	F (11.17±0.11), orig (11.13±0.16) > CF (9.70±0.23)
22	$F_{(2, 9)}$=12.838	p=0.002	F (11.36±0.11), orig (11.13±0.17) > CF (10.07±0.16)
23	$F_{(3, 11)}$=13.051	p=0.001	F (11.36±0.17), HR (11.32±NA), orig (11.27±0.11) > CF (10.03±0.23)
24	$F_{(3, 13)}$=15.807	p<0.001	F (11.40±0.10), HR (11.31±NA), orig (11.30±0.09) > CF (10.09±0.26)
25	$F_{(3, 11)}$=11.517	p=0.001	orig (11.43±0.08), F (11.42±0.10), HR (11.39±NA) > CF (10.49±0.02)
26	$F_{(3, 7)}$=13.608	p=0.003	orig (11.51±0.09), HR (11.49±NA), F (11.44±0.14) > CF (10.52±0.02)
28	$F_{(3, 7)}$=11.110	p=0.005	orig (11.59±0.08), F (11.57±NA), HR (11.52±NA) > CF (10.63±0.16)
34	$F_{(3, 2)}$=25.078	p=0.039	[F (12.01±NA), HR (11.71±NA) > CF (11.22±0.06)], [F > orig (11.54±0.05)]
35	$F_{(2, 2)}$=33.880	p=0.029	orig (12.08±0.03) > F (11.83±0.03), HR (11.64±NA)
potoroo			
21	$F_{(1, 6)}$=6.628	p=0.042	CF (7.86±0.13) > orig (7.42±0.04)

Values for mean foot length in potoroos followed the same three step trend (Fig.5.3.6). The mean results for cross-foster and original potoroos initially increased from less than half a centimeter (week 1, orig: 0.41cm ± 0.03) to ca. 2cm in the first eight weeks of pouch life (orig: 2.01cm ± 0.12, CF: 1.94cm ± 0.12). All transfer categories with available data reached a mean foot length of ca. 7cm at the end of the second, more rapid increase over the following nine

weeks leading up to pouch vacation (week 17, F: M=7.14cm, CF: 6.96cm ± 0.26, HR: M=6.61cm, orig: 6.59cm ± 0.08).

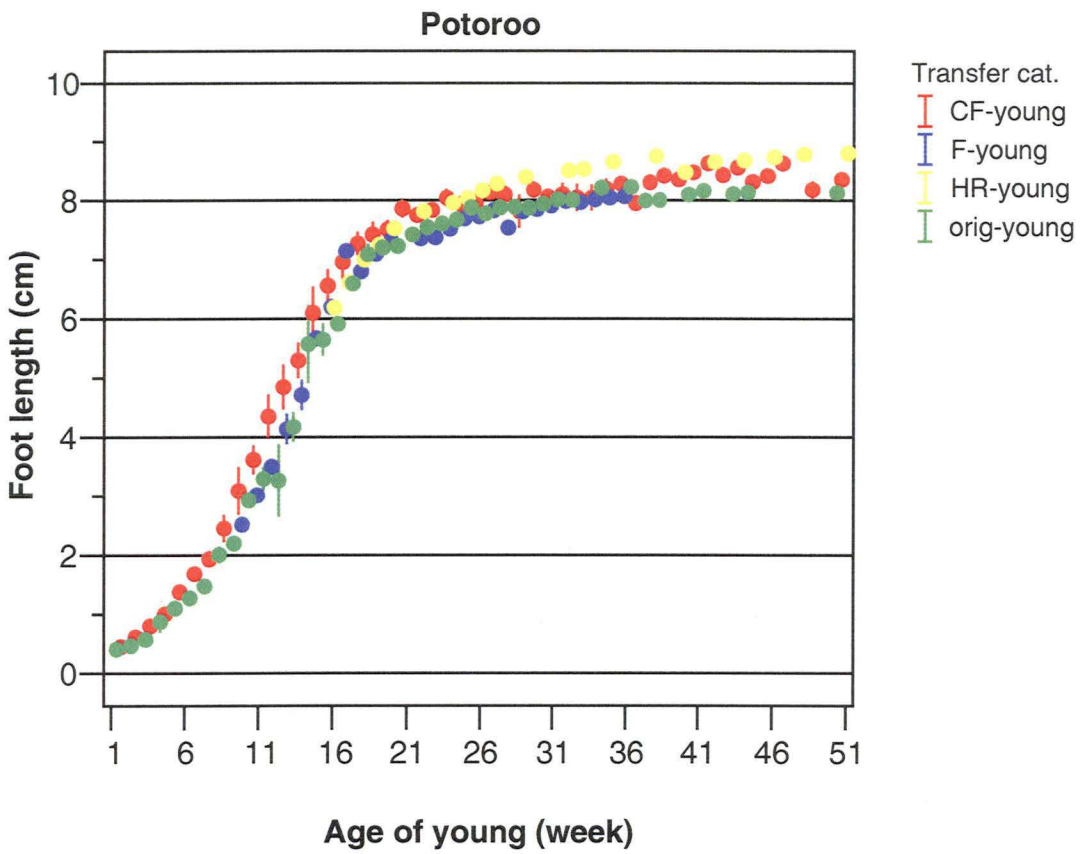

Fig.5.3.6: Changes in foot length (cm) of cross-fostered (red, N=7/155), fostered (blue, N=2/31), hand-reared (yellow, N=1/21) and original (green, N=31/122) potoroo young up to the age of 52 weeks. Error bars show Mean ± 1.0 SE.

Adult values (between 8 and 9cm) were produced for all categories in the third and steadier increase with subsequent plateau phase. The hand-reared potoroo appeared to have a growth advantage after week 29, but due to a lack of data any statistical significance of this difference could not be tested. Cross-foster potoroos had higher values between week 9 and 15, but a significant differences in mean foot length between the transfer groups could only be found in week 21 [$F_{(1, 6)}$=6.628, p=0.042] with cross-foster young differing significantly from original young.

5.3.1.4 Tail length

All transfer categories with available data showed an equal increase in mean tail length (Fig.5.6.7) from less than one centimetre (week 1, orig: 0.70cm ± 0.03) to ca. 7cm within the first ten weeks of pouch life (week 10, F: 7.89cm ± 0.35, orig: M=7.02cm, CF: 6.75cm ± 0.22). This was followed by a more rapid rise of results for foster young (25.37cm ± 1.29) and hand-reared bettongs (26.90cm ± 0.20) by week 20, whereas original young (21.38cm ± 2.45) and especially cross-foster bettongs (18.40cm ± 0.80) appeared to have fallen behind in their growth.

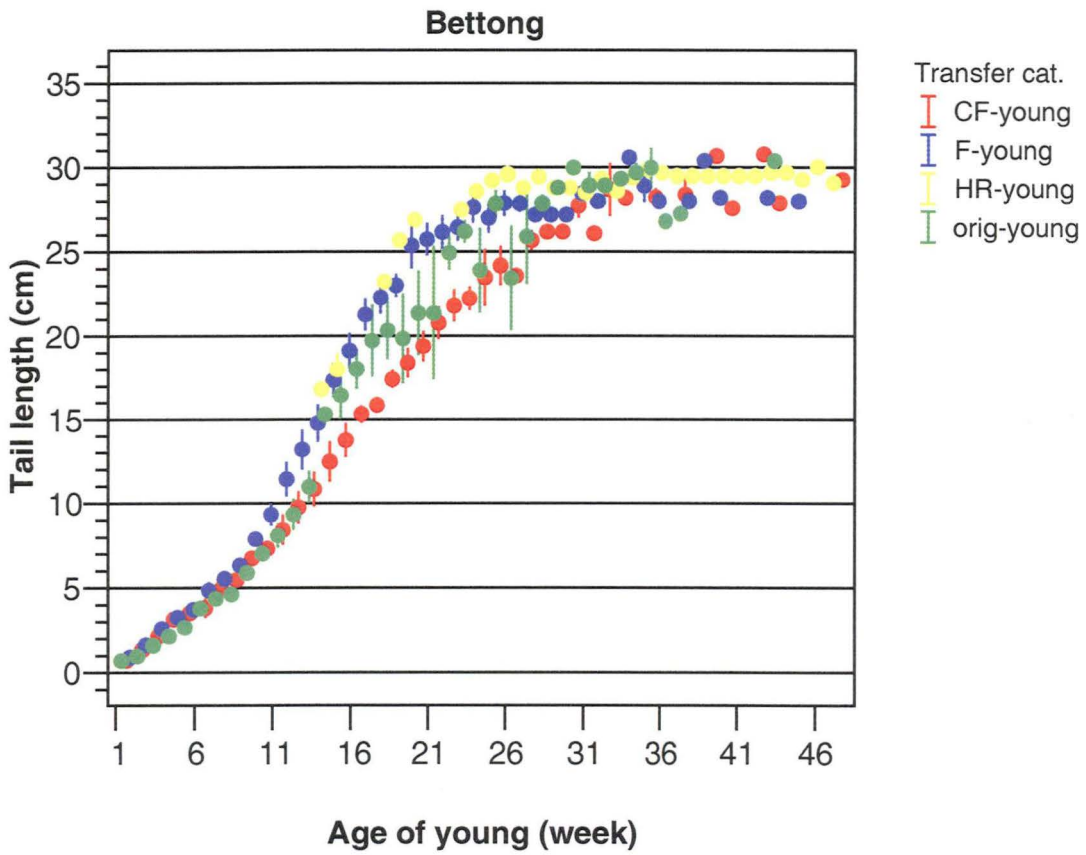

Fig.5.6.7: Changes in tail length (cm) of cross-fostered (red, N=5/86), fostered (blue, N=14/166), hand-reared (yellow, N=2/38) and original (green, N=67/165) bettong young up to the age of 52 weeks. Error bars show Mean ± 1.0 SE.

As with the growth measurements presented earlier, all bettong young reached the same mean adult tail length (ca. 30cm) in the final steady increase with following plateau phase. Since results for mean tail length in cross-foster bettongs

increased less rapidly, adult values were produced much later in life (week 33 compared to week 25 for hand-reared young). Significant differences in mean tail length between the different transfer groups were identified throughout lactation (Table 5.8).

Table 5.8: Significant results of the One way Anova test (F-ratio and p-value) for differences in tail length (cm) between the transfer groups of both species listed by weeks of age accompanied by group comparison results (LSD test); (CF=cross-foster, F=foster, HR=hand-reared, orig=original, >=significant difference, comma=no significant difference).

age	F-ratio	p-value	LSD results (Mean ± Standard error)
bettong			
8	$F_{(2, 13)}$=6.055	p=0.014	F (5.53±0.18) > orig (4.62±0.17)
14	$F_{(3, 14)}$=3.524	p=0.043	HR (16.83±0.12), orig (15.30±0.07), F (14.79±1.05) > CF (10.86±0.95)
17	$F_{(2, 13)}$=3.817	p=0.050	F (21.29±0.89) > CF (15.33±0.45)
18	$F_{(3, 13)}$=3.554	p=0.045	F (22.31±0.88) > CF (15.87±0.41)
22	$F_{(2, 7)}$=5.984	p=0.031	F (26.16±0.93), orig (24.93±0.93) > CF (20.80±0.90)
23	$F_{(3, 8)}$=8.383	p=0.007	HR (27.50±NA), F (26.45±0.75), orig (26.20±0.60) > CF (21.83±0.88)
potoroo			
10	$F_{(2, 10)}$=4.423	p=0.042	CF (6.43±0.49), orig (5.96±0.21) > F (4.74±0.12)
21	$F_{(1, 6)}$=6.147	p=0.048	CF (21.52±0.55) > orig (19.63±0.26)
22	$F_{(3, 7)}$=4.437	p=0.048	CF (20.92±0.34) > orig (19.36±0.32), HR (19.00±NA)

The results for mean tail length in potoroos (Fig.5.6.8) rose from just over half a centimetre (week 1, orig: 0.56cm ± 0.03) to ca. 4cm within the first nine weeks of pouch life for both cross-foster young (4.58cm ± 0.21) and original potoroo young (4.42cm ± 0.09). A more rapid increase occurred between week 10 and 20 with all categories reaching a mean foot length between ca. 19 and 20cm (week 20, CF: 20.14cm ± 0.67, F: M=19.35cm, HR: M=18.90cm, orig: 18.64cm ± 0.56). In this period a slight 'layer-effect' of values was present with cross-foster young producing higher results than foster young as well as foster young compared to hand-reared and original young.

Mean adult potoroo tail length values (ca. 25cm) were reached by young of most transfer categories in the final phase of steady increase as early as week 34 for foster potoroos (M=25.30cm) as well as week 40 for cross-foster potoroos (M=25.40cm) and hand-reared young (M=25.00cm). Equivalent results for original young were produced much later in life (week 75, M=24.50cm). Significant differences for mean tail length between the transfer groups were found in week 10 [$F_{(2, 10)}$=4.423, p=0.042], week 21 [$F_{(1, 6)}$=6.147, p=0.048] and week 22 [$F_{(3, 7)}$=4.437, p=0.048]. *Post-hoc* comparisons are summarised in Table 5.8.

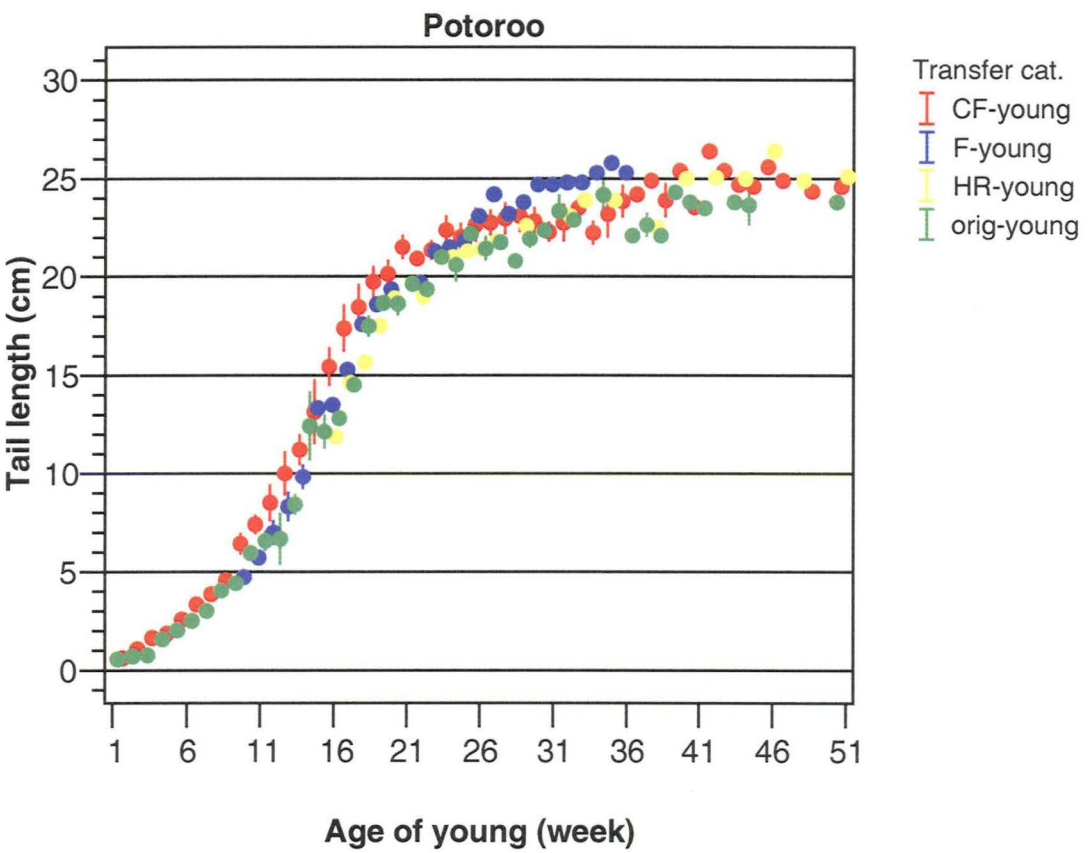

Fig.5.6.8: Changes in tail length (cm) of cross-fostered (red, N=7/155), fostered (blue, N=2/31), hand-reared (yellow, N=1/21) and original (green, N=30/121) potoroo young up to the age of 52 weeks. Error bars show Mean ± 1.0 SE.

5.3.1.5 Testis weight

A mostly constant increase in testis weight with age was found for all male bettong young in their first year of life (Fig.5.6.9). Results subsequently formed a plateau phase in week 42. There appeared to be a 'layer-effect' of values between week 9 and 24 with foster young producing higher testis weights compared to cross-foster young. This resulted in a series of significant differences in mean testis weight between the transfer groups during mid and late lactation as well as early sub-adulthood (Table 5.9). Layers began to merge from week 25 onwards.

Table 5.9: Significant results of the One way Anova test (F-ratio and p-value) for differences in mean testis weight (g) between the bettong transfer groups listed by weeks of age accompanied by group comparison results (LSD test); (CF=cross-foster, F=foster, orig=original, >=significant difference, >=significant difference, comma=no significant difference).

age	F-ratio	p-value	LSD results (Mean ± Standard error)
16	$F_{(2, 4)}$=15.965	p=0.012	orig (0.60±0.05) > F (0.37±0.02), CF (0.32±0.03)
17	$F_{(2, 5)}$=8.788	p=0.023	F (0.69±NA), orig (0.60±0.06) > CF (0.36±0.02)
19	$F_{(2, 2)}$=120.076	p=0.008	orig (0.81±NA) > F (0.49±NA), CF (0.44±0.01)
25	$F_{(2, 3)}$=20.268	p=0.018	F (1.33±0.03), orig (1.26±0.04) > CF (1.05±0.01)
26	$F_{(2, 1)}$=469.801	p=0.033	orig (1.62±NA) > F (1.29±NA) > CF (1.16±0.01)
36	$F_{(2, 1)}$=4589.756	p=0.010	F (4.63±NA) > CF (3.74±0.01) > orig (2.49±NA)

There was a greater degree of fluctuation in the results of original bettongs throughout the first year as well as for the other transfer categories during the final plateau phase. Original male bettong young reached a mean testis weight of 6.87g by week 43, which was representative of the adult males in this study.

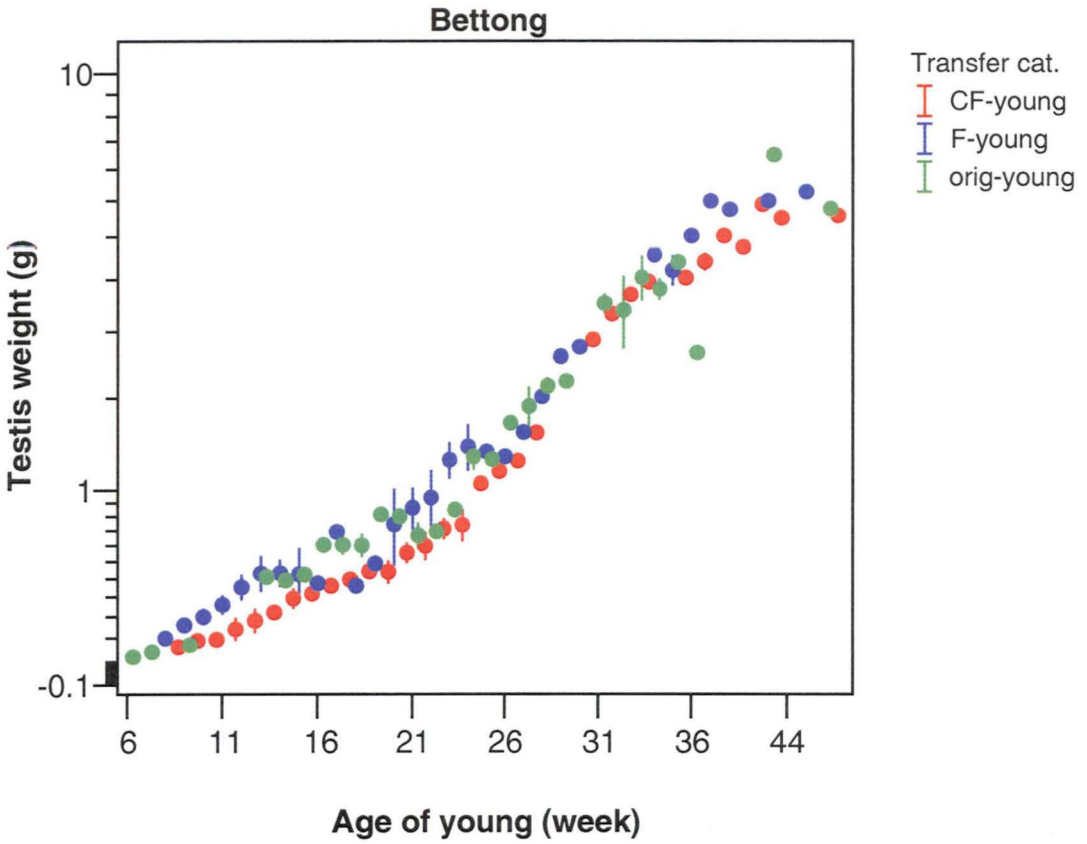

Fig.5.6.9: Logarithmic plot of testis weight (g) against age of young for cross-fostered (red, N= 3/57), fostered (blue, N=5/56) and original (green, N=20/49) bettong young. Error bars show Mean ± 1.0 SE.

The results for mean testis weight in potoroo young showed slight fluctuations within the transfer categories, but no distinct 'layer-effect' of values was found (Fig.5.6.10). The mean testis weight of cross-foster, hand-reared and original young increased steadily over time.

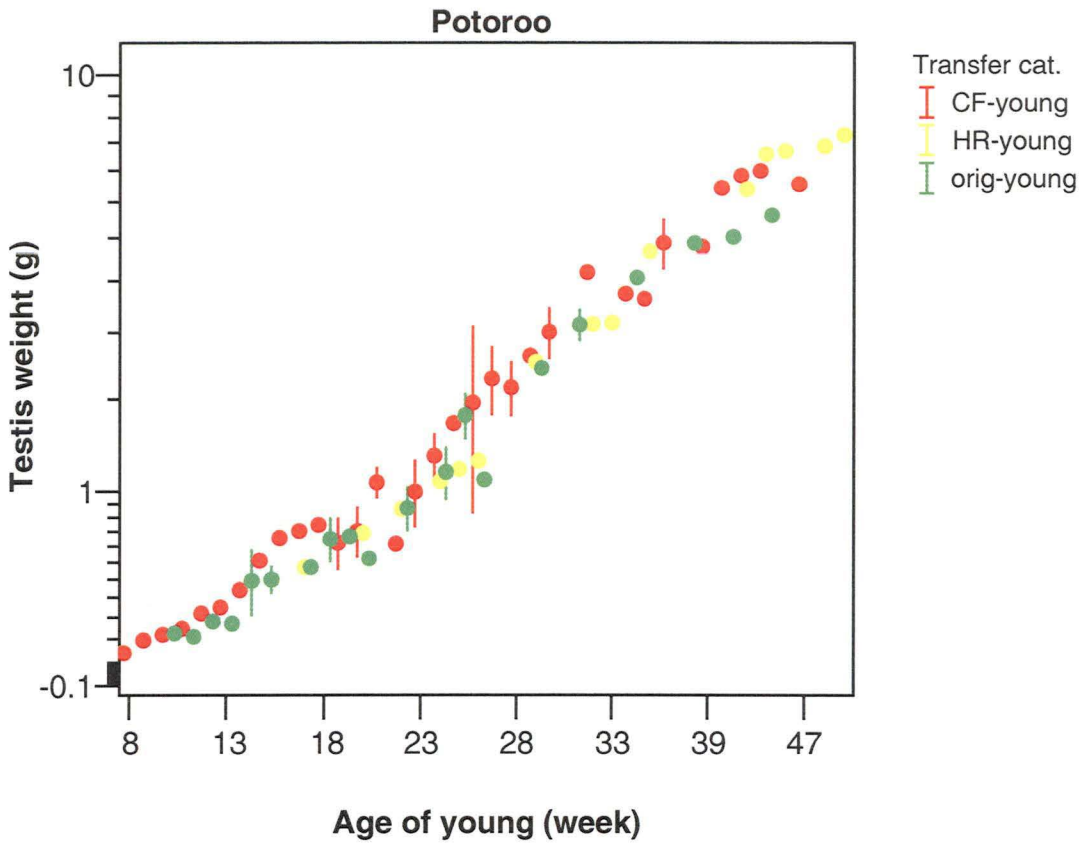

Fig.5.6.10: Logarithmic plot of testis weight (g) against age of young for cross-fostered (red, N=3/46), hand-reared (yellow, N=1/15) and original (green, N=8/36) potoroo young. Error bars show Mean ± 1.0 SE.

The mean adult potoroo testis weight for the animals in this study was slightly higher than for bettong males 7.4g. The hand-reared potoroo young was the first to reach adult levels in week 48 (M=7.18g), followed by cross-foster potoroos in week 57 (M=7.15g) and original potoroo young in week 75 (M=7.07g). No significant differences in mean testis weight were detected between the transfer groups.

5.3.1.6 Body condition

This index combined the already presented data for body weight and head length to give a better indication of the young's 'condition' (Fig.5.6.11). It indicated that cross-foster bettongs are in poorer condition for most of mid and late lactation (week 11 to 32).

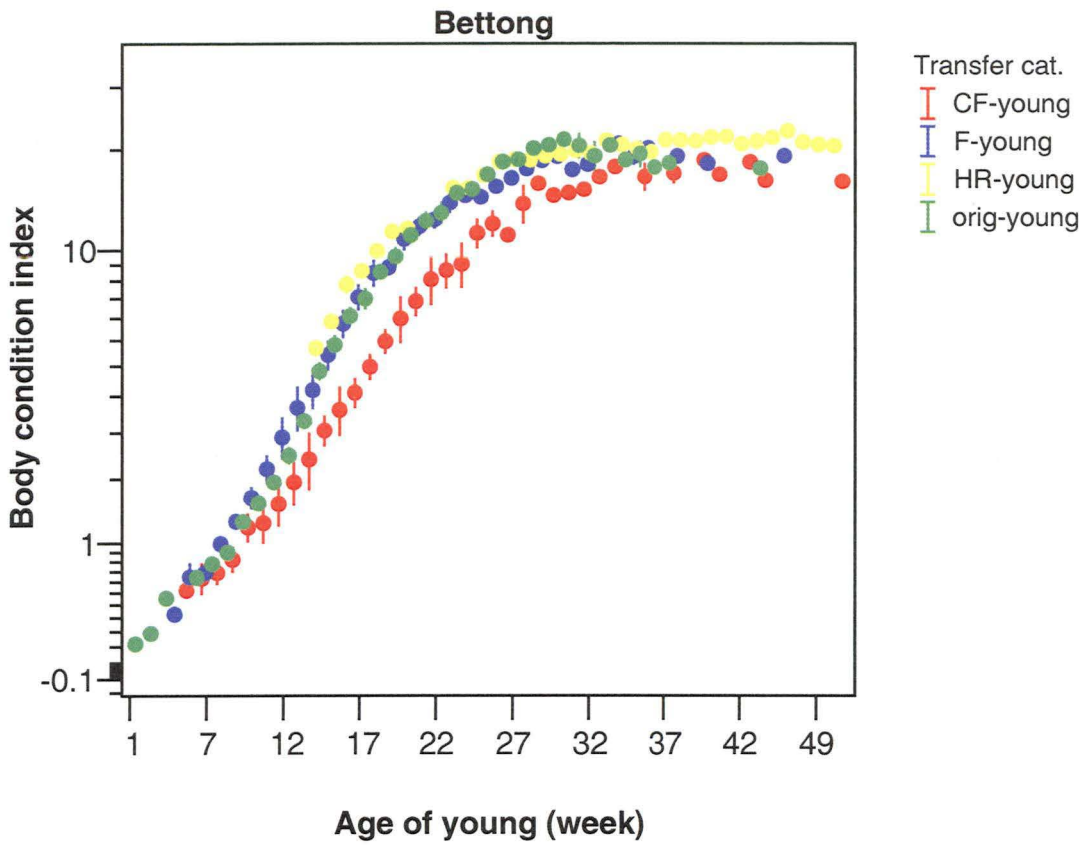

Fig.5.6.11: Logarithmic plot of body condition (measured as body weight/head length ratio in $g.mm^{-1}$) against age of young for cross-fostered (red, N=5/72), fostered (blue, N=14/156), hand-reared (yellow, N=1/46) and original (green, N=50/171) bettong young. Error bars show Mean ± 1.0 SE.

The mean body condition index of ca. '20' represented the maximum value for most transfer categories. Both hand-reared young (M=20.22) and original bettong young (M=19.58) reached maximum body condition levels in week 33, followed by foster young in week 34 (M=19.82). Values for cross-foster young remained lower throughout the first year and reached a maximum level by week 40 (M=17.90). Hand-reared bettongs appeared to be in better condition than other young close to pouch vacation (Fig.5.6.11, week 14 to 19) as well as in the final plateau phase (week 37 onwards). The significant differences in mean body condition between the transfer groups are summarised in Table 5.10.

Table 5.10: Significant results of the One way Anova test (F-ratio and p-value) for differences in mean body condition between the transfer groups for both species listed by weeks of age accompanied by group comparison results (LSD test); (CF=cross-foster, F=foster, HR=hand-reared, orig=original, >=significant difference, comma=no significant difference).

age	F-ratio	p-value	LSD results (Mean ± Standard error)
bettong			
8	$F_{(2, 10)}$=5.692	p=0.022	F (1.00±0.05) > CF (0.69±0.10)
9	$F_{(2, 14)}$=6.104	p=0.012	F (1.28±0.09), orig (1.27±0.06) > CF (0.83±0.12)
14	$F_{(3, 21)}$=5.732	p=0.005	HR (5.23±0.14), orig (4.43±0.25), F (3.87±0.48) > CF (2.26±0.52) and HR > F
15	$F_{(3, 25)}$=5.621	p=0.004	HR (6.27±0.18), orig (5.34±0.32), F (4.97±0.48) > CF (2.86±0.32)
16	$F_{(3, 22)}$=4.385	p=0.015	HR (8.05±0.42), orig (6.50±0.34), F (6.18±0.55) > CF (3.35±0.59)
17	$F_{(3, 19)}$=7.680	p=0.001	HR (8.79±0.21), F (7.38±0.58), orig (7.30±0.46) > CF (3.81±0.38)
18	$F_{(3, 14)}$=8.921	p=0.001	HR (10.02±NA), orig (8.71±0.26), F (8.66±0.69) > CF (4.58±0.38)
19	$F_{(3, 12)}$=11.718	p=0.001	HR (11.36±NA), orig (9.63±0.53), F (8.99±0.43) > CF (5.48±0.43)
20	$F_{(3, 14)}$=6.458	p=0.006	HR (11.58±0.03), orig (11.06±0.48), F (10.78±0.70) > CF (6.41±0.97)
21	$F_{(2, 11)}$=13.450	p=0.001	orig (12.15±0.62), F (11.71±0.62) > CF (7.19±0.64)
22	$F_{(2, 9)}$=5.651	p=0.026	orig (12.79±0.82), F (12.24±0.60) > CF (8.32±1.26)
23	$F_{(3, 11)}$=11.918	p=0.001	HR (15.01±NA), orig (14.51±0.49), F (13.63±0.77) > CF (8.82±0.94)
24	$F_{(3, 12)}$=10.745	p=0.001	HR (15.00±NA), orig (14.87±0.53), F (14.26±0.54) > CF (9.19±1.28)
25	$F_{(3, 11)}$=7.135	p=0.006	[orig (16.32±0.61), HR (16.24±NA), F (14.15±0.30) > CF (11.24±0.99)], [orig > F]
26	$F_{(3, 7)}$=9.239	p=0.008	{HR (17.67±NA), orig (17.65±0.75), F (15.13±0.08) > CF (11.95±0.92)], [orig > F]
27	$F_{(3, 8)}$=13.855	p=0.002	[HR (17.94±NA), orig (17.93±0.51), F (15.93±0.22) > CF (11.11±NA)], [orig > F]
28	$F_{(3, 6)}$=5.987	p=0.031	orig (19.24±0.62) > CF (13.57±1.56)
33	$F_{(2, 3)}$=10.374	p=0.045	HR (20.22±NA), orig (19.58±0.59) > CF (16.08±0.54)
potoroo			
21	$F_{(1, 6)}$=6.795	p=0.040	CF (6.74±0.31) > orig (5.68±0.06)

The potoroo results for mean body condition followed a similar trend (Fig.5.6.12) without forming distinct layers. Maximum levels in mean body condition were reached in week 40 for hand-reared young (M=11.97) and week 43 for cross-foster young (M=11.88). Values for original young remained lower in the plateau phase (week 43, M=9.78). Results for hand-reared young appeared

slightly higher from week 40 onwards compared to original young. Cross-foster potoroo young seemed to be in better condition during mid lactation (week 9 to 19) compared to other transfer categories, but a significant difference in mean body condition was only detected in week 21 [$F_{(1, 6)}$= 7.235, p=0.036], when results for cross-foster potoroo young were significantly higher than for original potoroo young.

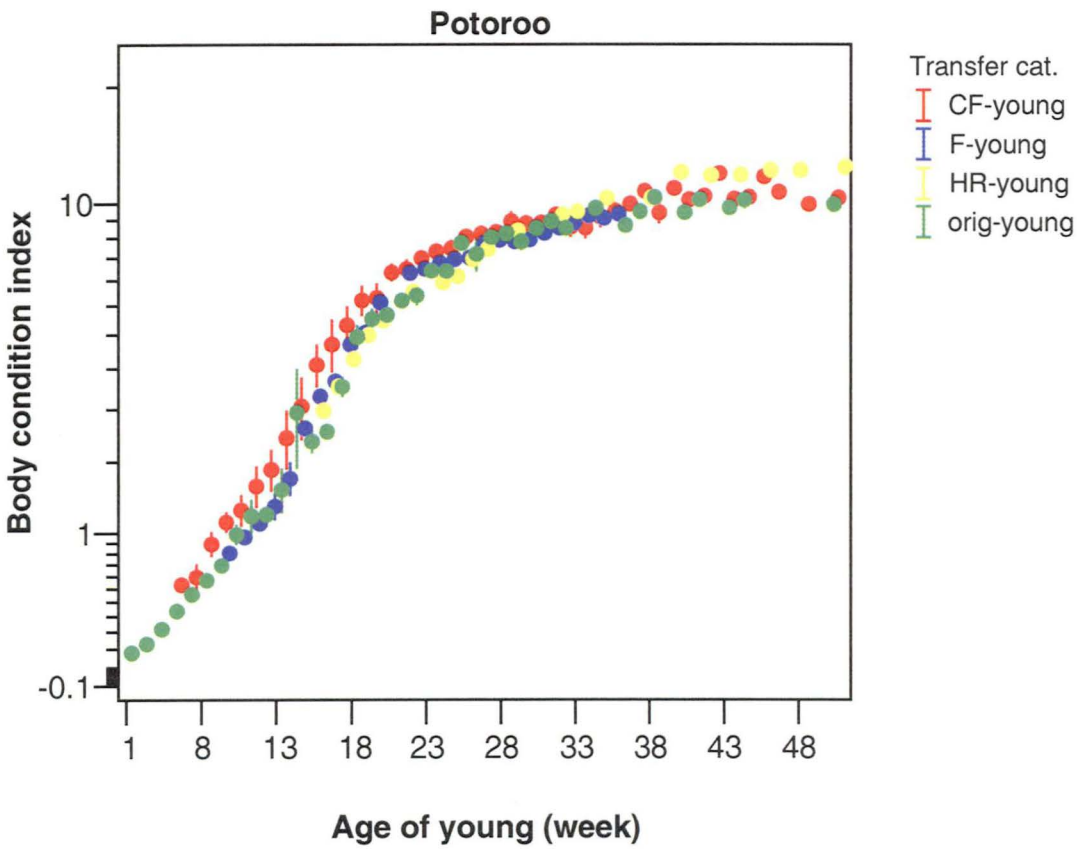

Fig.5.6.12: Logarithmic plot of body condition (measured as body weight/head length ratio in $g.mm^{-1}$) against age of young for cross-fostered (red, N=7/136), fostered (blue, N=2/31), hand-reared (yellow, N=1/21) and original (green, N=24/96) potoroo young. Error bars show Mean ± 1.0 SE.

5.3.2 Body measurements per transfer age difference

Since young of different ages were combined in the foster and cross-foster transfer categories of both species, these groups needed to be examined for differences between ages of each group. The growth results were initially divided by general age difference (same age, younger or older than the original

young, which used to inhabit their pouch). Subsequently the data was split by transfer age difference in weeks, provided sufficient mean values were present for performing statistical tests. The transfer age difference in weeks ranged from three weeks younger (-3) in weekly intervals to three weeks older (3).

5.3.2.1 Growth and general age difference

Significant differences between the age classes of bettong transfer groups were found across most of the measurement categories (Table 5.8). Mean body condition was used to illustrate the contrast between the age groups for foster bettong young, since most of the significant differences were detected in this transfer category (Fig.5.6.13).

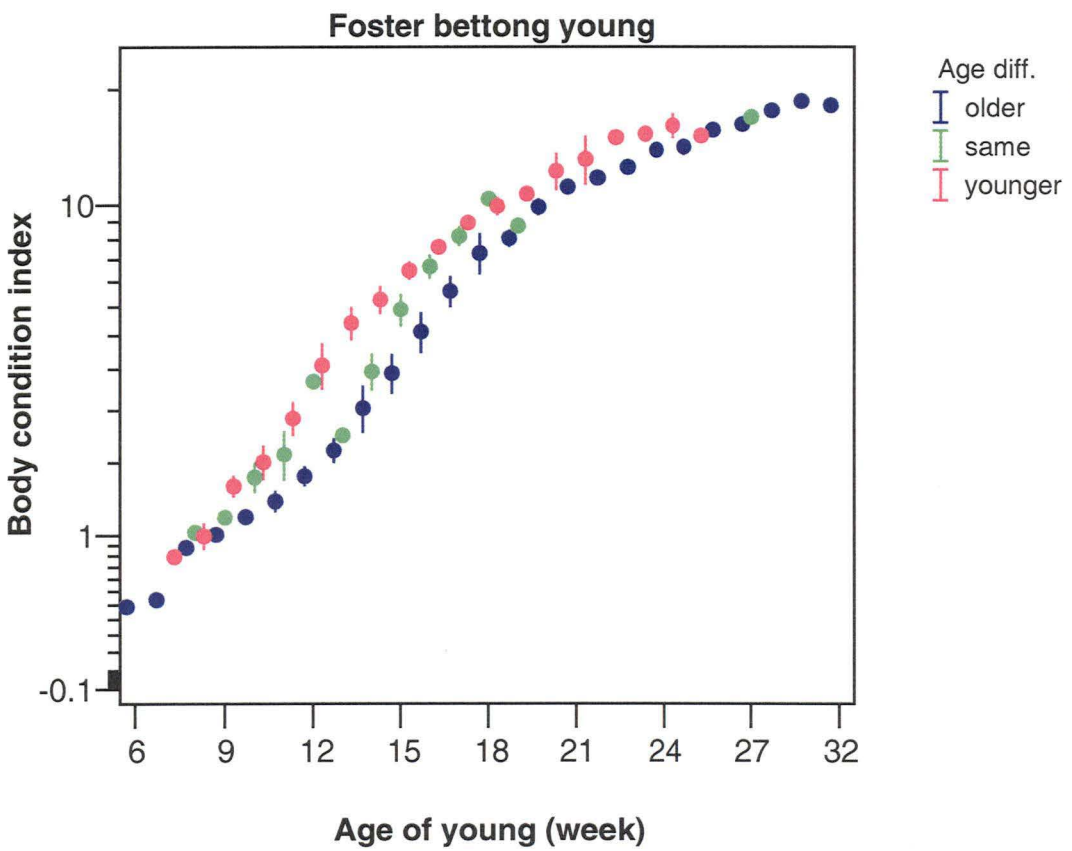

Fig.5.6.13: Logarithmic plot of body condition (measured as body weight/head length ratio in $g.mm^{-1}$) against age of young for older (blue, N=5/77), same age (green, N=5/34) and younger (purple, N=5/45) foster bettong young. Error bars show Mean ± 1.0 SE.

Younger foster bettongs appeared to be in better body condition between week 9 and 23 compared to older foster young. This provided younger foster bettongs with a useful advantage when becoming a YAF in terms of coping with environmental demands. Same aged foster bettongs mainly occupied the middle position. Significant differences between the age groups were found in all stages of lactation, but mainly close to pouch vacation (Table 5.11). The different age group layers began to merge from week 24 onwards.

Table 5.11: Significant results of the One way Anova test (F-ratio and p-value) for differences in various measurement categories between the general age classes of bettong transfer groups listed by weeks of age accompanied by group comparison results (LSD test); (Transfer category: CF=cross-foster, F=foster, HR=hand-reared, Y=younger, S=same age, O=older, >=significant difference, comma=no significant difference).

age	transfer	F-ratio	p-value	LSD results (Mean ± Standard error)
body condition (body mass/head length ratio, g.mm^{-1})				
9	F	$F_{(2, 4)}$=15.174	p=0.014	Y (1.58±0.13) > S (1.20±0.02), O (1.01±NA)
11	F	$F_{(2, 7)}$=4.929	p=0.046	Y (2.65±0.30) > O (1.38±0.12)
12	F	$F_{(2, 5)}$=10.357	p=0.017	Y (3.80±0.54) > O (1.71±0.12)
13	F	$F_{(2, 6)}$=16.644	p=0.004	Y (4.97±0.49) > S (2.34±NA), O (2.09±0.18)
14	F	$F_{(2, 5)}$=9.765	p=0.019	Y (5.75±0.46) > S (3.65±0.42), O (2.84±0.45)
15	F	$F_{(2, 7)}$=9.438	p=0.010	Y (6.85±0.32), S (5.40±0.51) > O (3.61±0.44)
16	F	$F_{(2, 6)}$=9.619	p=0.013	Y (7.87±0.09), S (7.01±0.46) > O (4.71±0.57)
17	F	$F_{(2, 5)}$=9.359	p=0.020	Y (9.05±0.28), S (8.38±0.43) > O (6.05±0.53)
body mass (g)				
7	F	$F_{(2, 1)}$=535.273	p=0.031	Y (25.47±0.26), S (22.89±NA) > O (11.20±NA)
9	F	$F_{(2, 4)}$=8.598	p=0.036	Y (64.57±4.80) > S (45.62±3.09), O (36.22±NA)
12	F	$F_{(2, 5)}$=11.120	p=0.014	Y (206.43±32.94), S (191.11±NA) > O (77.61±6.79)
13	F	$F_{(2, 6)}$=13.711	p=0.006	Y (288.85±34.93) > S (116.54±NA), O (102.03±12.40)
13	HR	$F_{(1, 11)}$=55.312	p<0.001	Y (253.63±14.34) > S (144.00±6.90)
14	F	$F_{(2, 5)}$=10.245	p=0.017	Y (352.24±40.91) > S (204.99±27.54), O (148.68±27.46)
14	HR	$F_{(1, 21)}$=60.420	p<0.001	Y (297.82±8.99) > S (195.31±7.74)
15	F	$F_{(2, 7)}$=11.278	p=0.006	Y (452.15±24.91) > S (324.02±34.26) > O (204.67±30.32)
15	HR	$F_{(1, 20)}$=121.642	p<0.001	Y (380.35±10.54) > S (213.07±9.08)
16	F	$F_{(2, 6)}$=13.089	p=0.006	Y (550.89±38.21), S (453.46±28.67) > O (281.14±38.18)
16	HR	$F_{(1, 20)}$=193.203	p<0.001	Y (492.57±11.59) > S (264.13±9.79)
17	F	$F_{(2, 6)}$=13.012	p=0.007	Y (629.10±26.06), S (579.22±42.08) > O (381.62±40.41)
17	HR	$F_{(1, 24)}$=102.265	p<0.001	Y (593.71±13.40) > S (323.89±15.28)

(Table 5.11 continued)

age	transfer	F-ratio	p-value	LSD results (Mean ± Standard error)
body mass (g)				
18	HR	$F_{(1, 22)}$=15.047	p=0.001	Y (684.96±9.55) > S (488.05±25.51)
21	F	$F_{(1, 4)}$=9.794	p=0.035	Y (1069.10±89.80) > O (836.68±33.28)
22	F	$F_{(1, 3)}$=10.220	p=0.049	Y (1233.30±NA) > O (935.80±41.62)
24	F	$F_{(1, 5)}$=7.054	p=0.045	Y (1407.40±127.49) > O (1109.27±29.56)
foot length (cm)				
5	CF	$F_{(1, 2)}$=20.638	p=0.045	Y (1.81±0.06) > S (1.23±NA)
12	F	$F_{(2, 5)}$=19.160	p=0.005	S (8.34±NA), Y (8.20±0.19) > O (6.19±0.27)
13	F	$F_{(2, 6)}$=12.780	p=0.007	Y (9.15±0.18) > S (7.27±NA), O (7.00±0.40)
15	HR	$F_{(1, 3)}$=17.357	p=0.025	Y (9.66±0.07) > S (9.00±NA)
27	F	$F_{(1, 2)}$=132.695	p=0.007	O (11.60±0.02) > S (11.06±NA)
head length (cm)				
12	F	$F_{(2, 5)}$=20.568	p=0.004	S (5.62±NA), Y (5.40±0.09) > O (4.52±0.12)
13	F	$F_{(2, 6)}$=8.194	p=0.019	Y (5.78±0.16) > O (4.85±0.19), S (4.74±NA)
14	F	$F_{(2, 5)}$=7.290	p=0.033	Y (6.12±0.23) > O (5.17±0.18)
15	F	$F_{(2, 7)}$=10.082	p=0.009	Y (6.60±0.07) > S (5.98±0.07), O (5.62±0.18)
16	F	$F_{(2, 6)}$=9.054	p=0.015	Y (7.00±0.38) > O (5.94±0.10)
17	F	$F_{(2, 5)}$=8.869	p=0.023	Y (7.12±0.21), S (6.91±0.14) > O (6.28±0.13)
tail length (cm)				
8	F	$F_{(2, 4)}$=7.951	p=0.040	Y (6.01±0.34) > O (4.74±NA)
12	F	$F_{(2, 5)}$=38.917	p=0.001	Y (14.20±0.32), S (12.79±NA) > O (9.05±0.45)
13	F	$F_{(2, 6)}$=34.663	p=0.001	Y (16.63±0.54) > S (10.75±NA), O (10.39±0.57)
16	F	$F_{(2, 6)}$=5.892	p=0.038	Y (22.98±0.33) > O (17.05±1.20)
23	F	$F_{(1, 2)}$=25.000	p=0.038	Y (27.70±0.00) > O (25.20±0.50)
testes weight (g)				
23	F	$F_{(1, 1)}$=208.333	p=0.044	Y (1.57±NA) > O (1.10±0.02)

Mean body mass of hand-reared bettong young was chosen as a second example to explore a possible advantage of transferred over originally hand-reared young in regards to different age groups. These animals were not divided into different age groups, since they were transferred from their original mothers to a human 'mother' without displacing a different aged young. One bettong foster young was hand-reared as part of this study after being rejected by its foster mother. The data for this particular young is labeled in Figure 5.6.14 with the age difference it used to have as a foster young.

The results for the younger hand-reared bettong were slightly higher throughout its first year compared to the originally hand-reared young (labeled as same). The difference appeared to be more pronounced in the time following pouch vacation (week 15 to 19) as well as weaning (week 27 onwards). Significant differences between the age groups (Table 5.11) should not be overrated, since the age category 'younger' consisted of only one animal.

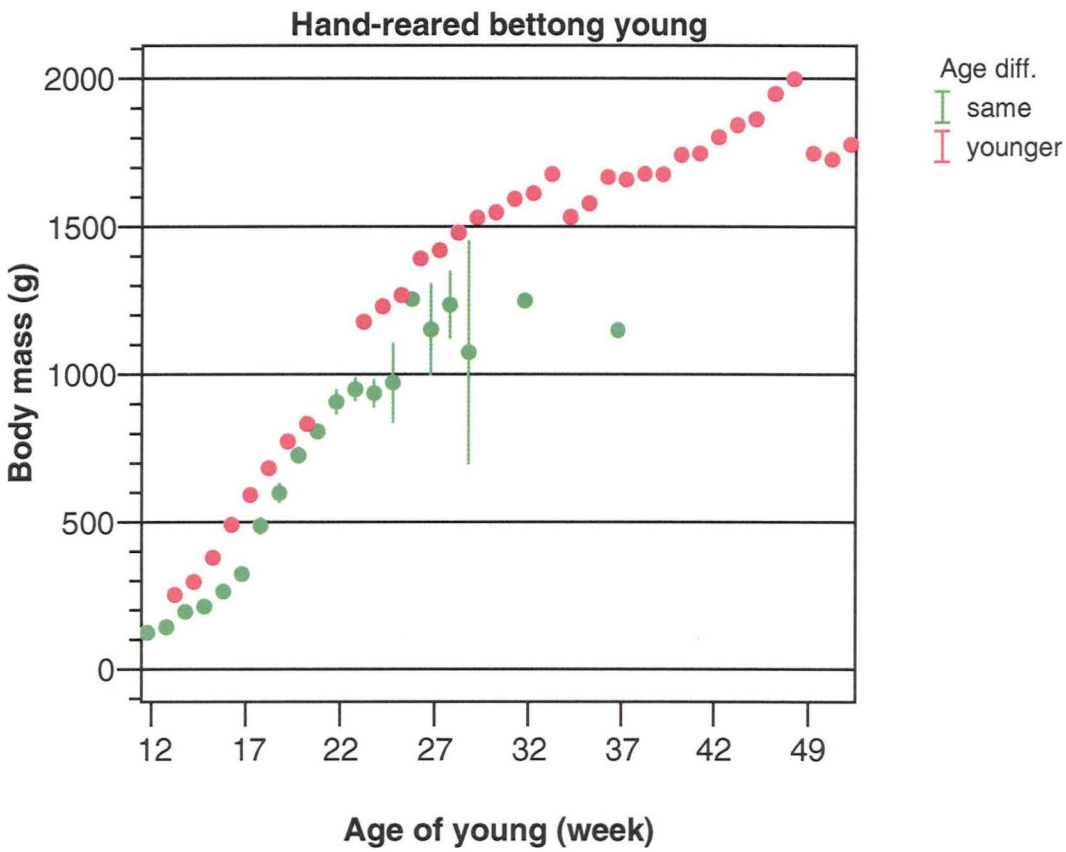

Fig.5.6.14: Changes in body mass (g) of same age (green, N=9/198) and younger (purple, N= 1/67) hand-reared bettong young up to the age of 52 weeks. Error bars show Mean ± 1.0 SE.

5.3.2.2 Growth and transfer age difference in weeks

The TAD in weeks gave an insight into the consistency of growth differences across all age groups. Significant differences between the age groups are summarised in Table 5.12. The described layers for general age difference in mean body condition of foster bettong young were still apparent on the more detailed level of transfer age difference in weeks (Fig.5.6.15).

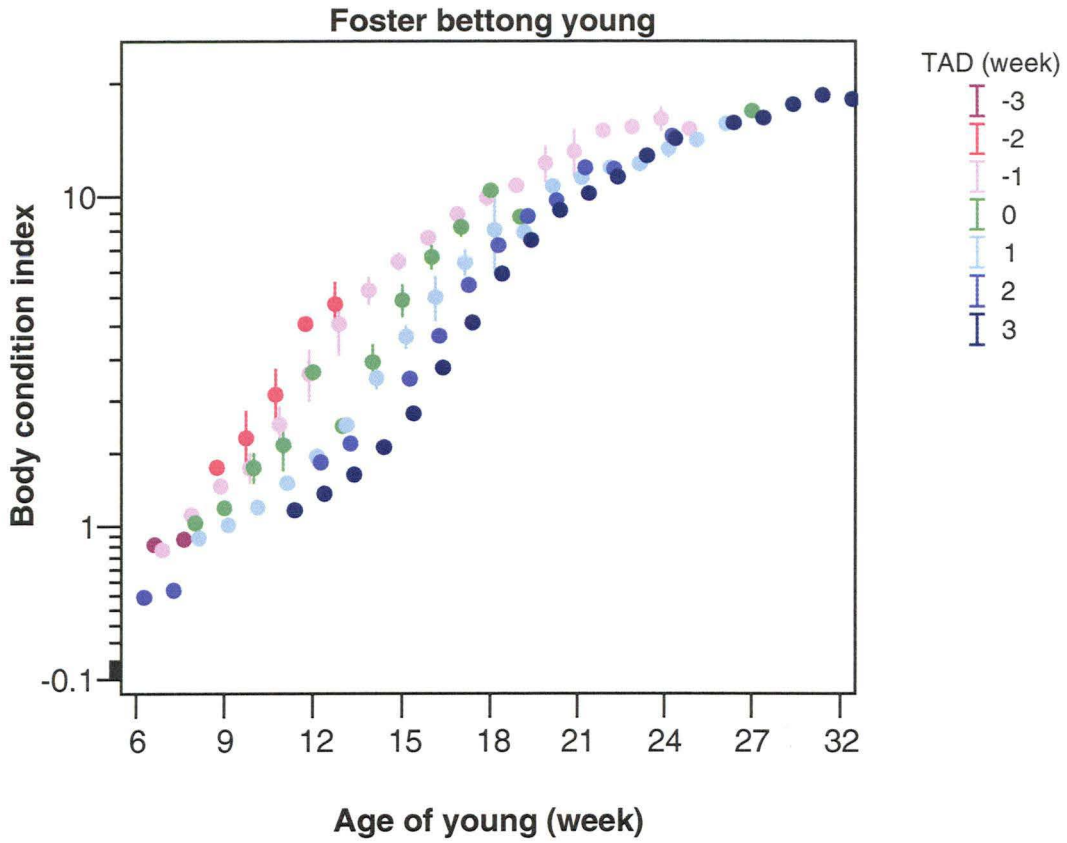

Fig.5.6.15: Logarithmic plot of body condition (measured as body weight/head length ratio in $g.mm^{-1}$) against age of young for three weeks younger (-3, dark purple, N=1/2), two weeks younger (-2, medium purple, N=2/9), one week younger (-1, light purple, N=2/34), same age (0, green, N=5/34), one week older (1, light blue, N=2/34), two week older (2, medium blue, N= 2/15) and three week older (3, dark blue, N=1/28) foster bettong young. Error bars show Mean ± 1.0 SE.

The advantage of younger over older transfer animals was especially apparent at the time of pouch vacation (15 weeks) – the younger the foster bettong the higher the mean body condition ratio.

Table 5.12: Significant results of the One way Anova test (F-ratio and p-value) for differences in various measurement categories between the age classes for transfer age difference in weeks of bettong and potoroo transfer groups listed by weeks of age accompanied by group comparison results (LSD test); (Transfer category: CF=cross-foster, F=foster, HR=hand-reared, (guide): number displays transfer age difference in weeks, negative values=younger, positive values= older, e.g. '–1' = one week younger, >=significant difference, comma=no significant difference).

age	transfer	F-ratio	p-value	LSD results (Mean ± Standard error)
bettong				
foot length (cm)				
13	F	$F_{(5, 3)}$=14.012	p=0.027	[-1 (9.19±0.34), -2 (9.10±0.26) > 1 (7.58±0.26), 0 (7.27±NA), 2 (6.90±NA), 3 (5.94±NA)], [1 > 3]
14	F	$F_{(3, 4)}$=8.242	p=0.035	-1 (9.86±0.24), 0 (8.75±0.31), 1 (8.56±0.40) > 3 (6.81±NA)
15	F	$F_{(4, 5)}$=6.433	p=0.033	-1 (10.17±0.24), 0 (9.81±0.22), 1 (9.50±0.35) > 3 (7.74±NA)
15	HR	$F_{(1, 3)}$=17.357	p=0.025	-2 (9.66±0.07) > 0 (9.00±NA)
16	F	$F_{(4, 4)}$=9.450	p=0.026	-1 (10.45±0.16), 0 (10.39±0.18), 1 (10.03±0.11) > 3 (8.75±NA)
tail length (cm)				
6	CF	$F_{(2, 1)}$=1467.000	p=0.018	-2 (4.09±NA) > -1 (3.49±0.01) > 0 (3.01±NA)
8	F	$F_{(3, 3)}$=30.966	p=0.009	-3 (6.35±NA) > -1 (5.67±NA), 0 (5.49±0.06) > 1 (4.74±NA)
13	F	$F_{(5, 3)}$=10.577	p=0.040	-1 (16.70±1.00), -2 (16.55±0.85) > 1 (11.14±0.86), 0 (10.75±NA), 2 (9.91±NA), 3 (9.39±NA)
14	F	$F_{(3, 4)}$=6.909	p=0.046	-1 (18.75±0.05) > 1 (14.35±0.75), 0 (13.82±1.22), 3 (10.64±NA)
20	F	$F_{(3, 1)}$=985.681	p=0.023	[-1 (27.33±0.08) > 2 (25.00±NA) > 3 (20.50±NA)], [1 (26.70±NA) > 3]
head length (cm)				
12	F	$F_{(5, 2)}$=21.084	p=0.046	[0 (5.62±NA) > 1 (4.71±0.10), 3 (4.42±NA), 2 (4.26±NA)], [-2 (5.55±NA), -1 (5.33±0.10) > 1, 3, 2]
14	F	$F_{(3, 4)}$=9.518	p=0.027	[-1 (6.12±0.23) > 1 (5.35±0.04), 3 (4.81±NA)], [0 (5.60±0.11) > 3]
15	F	$F_{(4, 5)}$=8.278	p=0.020	[-1 (6.60±0.07) > 0 (5.98±0.07), 1 (5.77±0.28), 2 (5.71±NA0, 3 (5.21±NA)], [0 > 3]
17	CF	$F_{(1, 1)}$=161.333	p=0.050	-2 (5.90±NA) > -1 (5.68±0.01)
body mass (g)				
13	HR	$F_{(1, 11)}$=55.312	p<0.001	-2 (253.63±14.34) > 0 (144.00±6.90)
14	F	$F_{(3, 4)}$=8.922	p=0.030	-1 (352.24±40.91) > 0 (204.99±27.54), 1 (175.10±12.96), 3 (95.83±NA)
14	HR	$F_{(1, 21)}$=60.420	p<0.001	-2 (297.82±8.99) > 0 (195.31±7.74)
15	F	$F_{(4, 5)}$=6.882	p=0.029	[-1 (452.15±24.91) > 1 (249.16±29.82), 2 (186.02±NA), 3 (134.35±NA)], [0 (324.02±34.26) > 3]
15	HR	$F_{(1, 20)}$=121.642	p<0.001	-2 (380.35±10.54) > 0 (213.07±9.08)
16	F	$F_{(4, 4)}$=10.201	p=0.022	[-1 (550.89±38.21) > 1 (335.22±46.28), 2 (254.59±NA), 3 (199.54±NA)], [0 (453.46±28.67) > 2, 3]

(Table 5.12 continued)

age	transfer	F-ratio	p-value	LSD results (Mean ± Standard error)
bettong				
body condition (body mass/head length ratio, g.mm^{-1})				
16	HR	$F_{(1, 20)}$=193.203	p<0.001	-2 (492.57±11.59) > 0 (264.13±9.79)
17	F	$F_{(4, 4)}$=11.932	p=0.017	[-1 (629.10±26.06) > 1 (439.82±42.52), 2 (358.32±NA), 3 (288.53±NA)], [0 (579.22±42.08) > 2, 3]
17	HR	$F_{(1, 24)}$=102.265	p<0.001	-2 (593.71±13.40) > 0 (323.89±15.28)
18	HR	$F_{(1, 22)}$=15.047	p=0.001	-2 (684.96±9.55) > 0 (488.05±25.51)
23	CF	$F_{(1, 1)}$=227.712	p=0.042	-2 (815.50±NA) > -1 (567.20±9.50)
9	F	$F_{(3, 3)}$=60.204	p=0.004	-2 (1.70±NA) > -1 (1.45±NA) > 0 (1.20±0.02) > 1 (1.01±NA)
14	F	$F_{(3, 4)}$=9.801	p=0.026	-1 (5.75±0.46) > 0 (3.65±0.42), 1 (3.27±0.22), 3 (1.99±NA)
15	F	$F_{(4, 5)}$=6.266	p=0.035	[-1 (6.85±0.32) > 1 (4.30±0.30), 2 (3.26±NA), 3 (2.56±NA)], [0 (5.40±0.51) > 3]
16	F	$F_{(4, 4)}$=8.410	p=0.031	[-1 (7.87±0.09) > 1 (5.50±0.72), 2 (4.32±NA), 3 (3.51±NA)], [0 (7.01±0.46) > 2, 3]
17	F	$F_{(4, 3)}$=13.217	p=0.030	[-1 (9.05±0.28) > 1 (6.79±0.49), 2 (5.93±NA), 3 (4.70±NA)], [0 (8.38±0.43) > 2, 3]
23	CF	$F_{(1, 1)}$=11739.593	p=0.006	-2 (10.70±NA) > -1 (7.89±0.02)
potoroo				
foot length (cm)				
12	CF	$F_{(1, 1)}$=1160.333	p=0.019	1 (5.05±NA) > 2 (4.01±0.02)
14	CF	$F_{(2, 4)}$=7.004	p=0.049	1 (6.16±0.43) > 3 (4.72±0.03)
18	CF	$F_{(2, 3)}$=44.375	p=0.006	1 (7.71±0.02) > 2 (7.31±0.12) > 3 (6.78±0.00)
tail length (cm)				
14	CF	$F_{(2, 4)}$=11.973	p=0.020	1 (13.49±0.44) > 3 (9.54±0.12)
head length (cm)				
18	CF	$F_{(2, 3)}$=28.092	p=0.011	1 (7.68±0.02) > 2 (7.06±0.01), 3 (6.64±0.17)
20	CF	$F_{(2, 2)}$=88.632	p=0.011	1 (8.14±NA) > 2 (7.68±0.05) > 3 (7.14±0.05)
30	CF	$F_{(2, 2)}$=56.524	p=0.017	1 (9.00±0.06) > 2 (8.48±0.03), 3 (8.24±NA)
body mass (g)				
39	CF	$F_{(2, 2)}$=142.085	p=0.007	3 (923.77±3.14) > 1 (852.80±NA), 2 (830.00±NA)

Changes in mean head length for cross-foster potoroo young were chosen as the second example for varying growth rates in regards to different transfer ages in weeks (Fig.5.6.16), since differences between the age groups were not apparent on a general age difference level.

There was a distinct 'layer-effect' in results for the one to three weeks older cross-foster potoroo young between the weeks 13 and 33. One week older cross-foster potoroo young produced the highest results for mean head length per age during that period, followed by two and three weeks older young. The mean head length for one week older cross-foster potoroos at time of pouch vacation was 7.49cm. An equivalently high value for three weeks older young was produced six weeks later (week 23: 7.47cm ± 0.15). Significant differences for mean head length in cross-foster potoroo young between the age groups were found in week 18, 20 and 30 (Table 5.12).

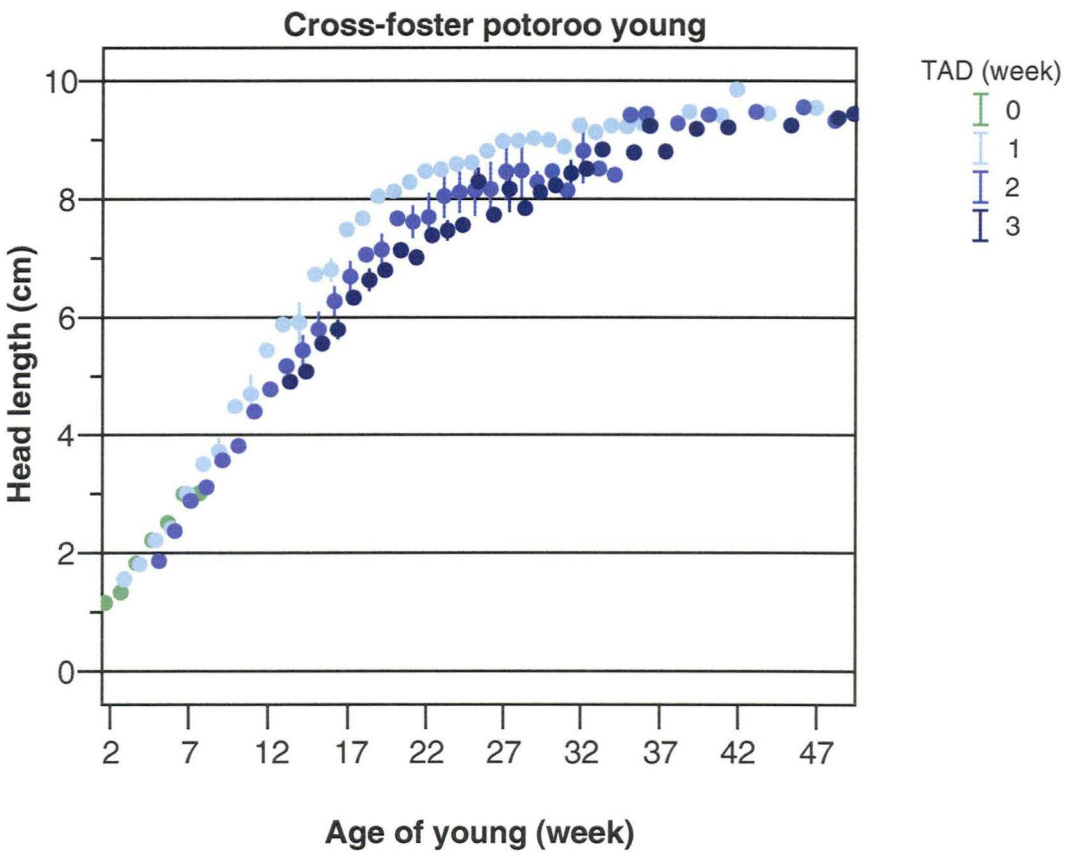

Fig.5.6.16: Changes in head length (cm) of same age (green, N=1/7), one week older (1, light blue, N=2/50), two weeks older (2, medium blue, N=2/58) and three weeks older (3, dark blue, N=2/40) cross-foster potoroo young up to the age of 52 weeks. Error bars show Mean ± 1.0 SE.

5.3.3 Development

The development was analysed in conjunction with the growth of young to determine possible impacts of pouch young transfers. The developmental stages of a series of body features were explored for both species in regards of transfer category and TAD (general and weekly level).

Table 5.13: Significant results of the One way Anova test (F-ratio and p-value) for differences in development of various body features for different transfer categories and age classes (general/ weekly difference) of bettong young listed by weeks of age accompanied by group comparison results (LSD test); (CF=cross-foster, F=foster, orig=original, general age diff.: S=same age, Y= younger, O=older, weekly age diff.: number displays TAD in weeks, negative values=younger, positive values=older, e.g. '–1' = 1 week younger, >=significant difference, comma=no significant difference).

age	F-ratio	p-value	LSD results (Mean ± Standard error)
development criteria for bettong transfer categories			
hair			
15	$F_{(2, 12)}$=5.631	p=0.019	orig (4.0±0.0), F (3.4±0.2) > CF (2.7±0.3)
locomotion			
10	$F_{(2, 15)}$=3.707	p=0.049	F (2.8±0.1) > orig (2.0±0.0)
11	$F_{(2, 14)}$=5.353	p=0.019	F (2.9±0.1) > orig (2.2±0.2)
mouth			
10	$F_{(2, 15)}$=6.641	p=0.009	CF (4.0±0.0), F (3.9±0.1) > orig (3.3±0.3)
11	$F_{(2, 14)}$=7.412	p=0.006	CF (4.0±0.0), F (3.9±0.1) > orig (3.3±0.3)
pigment			
5	$F_{(2, 8)}$=8.727	p=0.010	CF (2.0±0.0), F (2.0±0.0) > orig (1.2±0.2)
9	$F_{(2, 14)}$=4.188	p=0.038	F (3.3±0.4) > orig (2.3±0.3), CF (2.0±0.0)
10	$F_{(2, 13)}$=5.688	p=0.017	F (4.4±0.2) > CF (3.0±0.6), orig (3.0±1.0)
11	$F_{(2, 11)}$=6.875	p=0.012	F (4.8±0.2), orig (4.7±0.3) > CF (3.0±1.0)
development criteria for F-bettongs/general TAD			
eyes			
11	$F_{(2, 7)}$=6.300	p=0.027	Y (1.8±0.3) > S (1.0±0.0), O (1.00±0.0)
hair			
13	$F_{(2, 6)}$=10.778	p=0.010	Y (3.8±0.3) > O (2.3±0.3), S (2.0±NA)
pouch life			
13	$F_{(2, 6)}$=13.889	p=0.006	Y (2.3±0.3) > O (1.0±0.0), S (1.0±NA)
development criteria for F-bettongs/TAD in weeks			
hair			
14	$F_{(3, 4)}$=8.000	p=0.036	[-1 (4.0±0.0) > 0 (3.0±0.0), 3 (2.0±NA)], [1 (3.5±0.5) > 3]

Bettong foster young appeared to develop slightly faster than cross-foster and original bettong young in most of the chosen body features. Younger foster bettongs had an advantage over same aged young and older young (Table 5.13) on the level of general and weekly transfer age difference. Cross-foster bettongs being one week younger showed more deficits in development compared to two weeks younger cross-foster bettongs (Fig.5.6.17). Cross-foster potoroos showed more advanced development than original potoroos. Cross-foster potoroos being one and/or two weeks older had an advantage over three weeks older cross-foster potoroo young on the transfer age difference level. No significant differences in development between the various transfer groups and age classes were found for potoroos.

The transfer animals shown in Figure 5.6.17 displayed various degrees of differences in growth and development. No obvious difference was noticeable for the foster young in pair 'A' with a transfer age difference of one week. Although pair 'B' had the same transfer age difference, the younger animal had a clear advantage in both growth and development compared to its older foster partner. The older one was much smaller, had gained less weight and was covered by fine fur. The younger one on the contrary was already covered by guard hair and was much larger in seize and in better body condition.

The cross-foster animals in pair 'C' with a transfer age difference of two weeks appeared to be at a similar developmental stage, however the potoroo was much larger in seize and in better body condition. In cross-foster pair 'D' with a transfer age difference of only one week the potoroo was clearly advanced in growth and development (fully furred, good body condition) while the bettong was too small for its age with fine hair only commencing to grow.

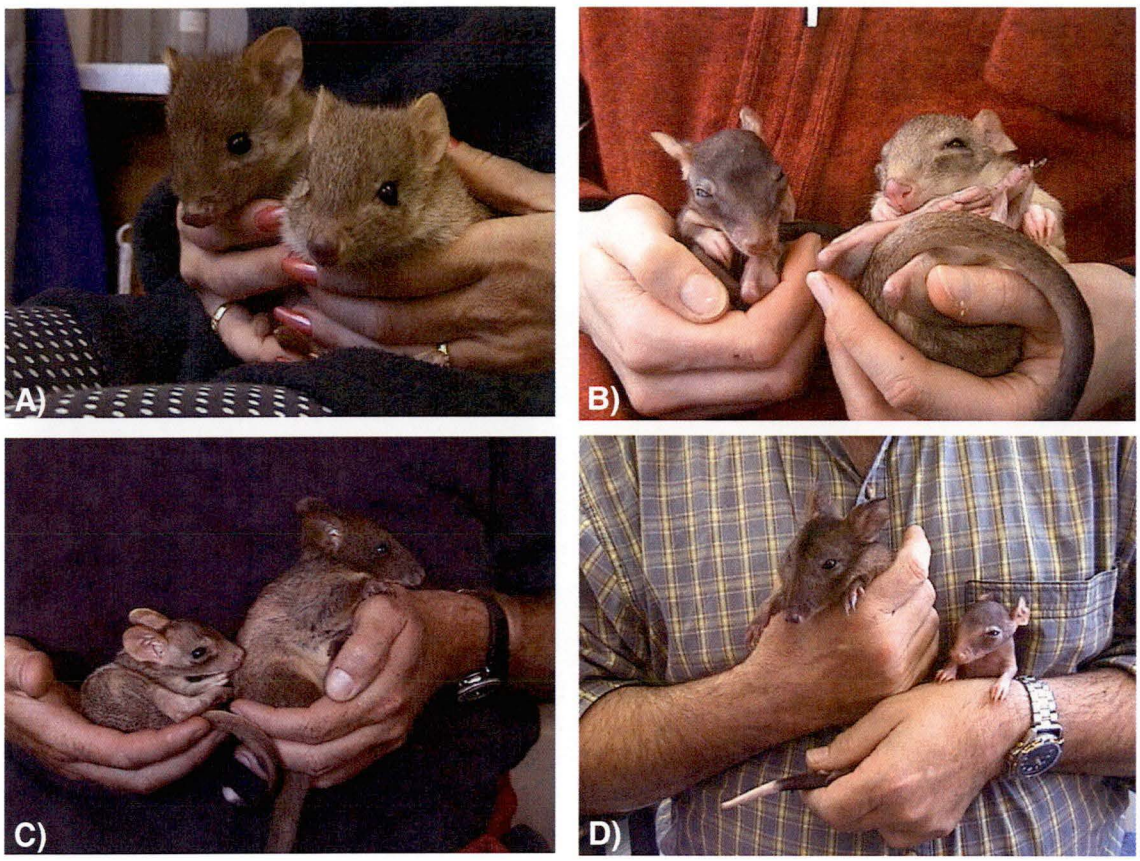

Fig.5.6.17: Foster and cross-foster young with various age differences, A) foster bettong young with a transfer age difference (TAD) of 1 week, older one (17 weeks) left, B) foster bettong young with a TAD of 1 week, older one (14 weeks) left, C) cross-foster young with a TAD of 2 weeks, older one (potoroo, 17 weeks) right, D) cross-foster young with a TAD of 1 week, older one (potoroo, 15 weeks) left.

The cross-foster bettong (pair 'D') was also less developed compared to the older foster young from pair 'B' (left), although both were of similar age. The cross-foster potoroo, however, appeared to be at an equivalent stage of growth and development compared to the cross-foster potoroo from pair 'C', although the latter one was two weeks older in comparison.

5.4 Discussion

The collected growth measurements corresponded well with results of other authors for the same species (Rose 1984, Bryant 1989). All obtained body measurements indicated a growth advantage for cross-fostered potoroos and a growth disadvantage for cross-fostered bettong young compared to the other transfer categories. The latter were lacking in muscle growth as PY and early

YAFs, which might indicate a protein deficiency, since protein is essential for tissue growth and development. The proportion of milk energy provided by protein can be used as an index of growth resources for the young (Oftedal 1980). Therefore it has to be assumed that the protein concentrations in potoroo milk do not satisfy the growth related needs of cross-foster bettongs (TAD between 0 and –3). Delayed growth by undernutrition was balanced once the young had access to solid food, but led to reduced adult size for one cross-foster bettong. The latter result corresponded well with the effects of undernutrition on growing mammals described by McCane and Widdowson (1974). They investigated undernutrition during various growth stages of piglets and showed that long-term effects, such as reduced adult size, were dependent on the duration and severity of the energy restriction as well as the stage of growth at which it occurred. Undernutrition that occurred early in life for prolonged periods led to reduced adult pig size.

These differences in growth between the transfer groups were most pronounced in the period between pouch vacation and weaning. Fostered bettong young had a slight advantage over original bettong young depending on the TAD. Improved growth rates appeared to be related to a younger age of transferees. However, the 'time window' of TAD for successful pouch young transfer appeared to be restricted to a maximum of three weeks. Young with a TAD of three weeks experienced growth related problems. The loss of three of the younger transferees might have been caused by an inability to digest the milk, which was produced for a more mature young. The growth of the three weeks older bettong fostered young appeared to be retarded and although he matured and fathered several young successfully, his behaviour (e.g. slow reaction time, established no dominance over his young, showed no defensive behaviour when attacked by his son) gave reason to assume that some brain damage possibly occurred in early development due to insufficient nutrition.

Merchant and Sharman (1966) showed that the successful transfer of pouch young into pouches of virgin recipient mothers is possible. Although this approach is very promising from an ethical point of view, since no original pouch

young has to be sacrificed, it automatically places the transferred young into a disadvantaged position due to the TAD (unless transferred at time of birth or placed in a pouch of a species with a higher growth rate). Inexperience of primiparous mothers may also compromise the successful rearing of the transferred young.

The results for animals with a TAD of one week indicated that the slight change in milk composition was affecting growth and development depending on the individual ability of the young to utilize the provided nutrients. While some young appeared to have a normal growth rate during consumption of more advanced milk, others were slightly obese. This growth advantage, however, disappeared after pouch vacation, when young increasingly consumed solid food in addition to milk and energy expenditure increased. None of the latter animals were lost during the study opposed to findings of Merchant and Sharman (1966) and Clark (1968). The latter author stressed the fact that optimal growth is not necessarily always maximal growth, since a very rapid growth rate may be harmful to the young. Therefore it is important to distinguish between "plump" and severely obese to ensure the long-term well-being of the transferred young.

Smith (1989) also found accelerated growth and development in young drinking more advanced milk. She could clearly see subcutaneous fat and described the appearance of the young as "plump". This surplus of energy in slightly obese animals in the present study (e.g. Fig.5.6.17B/right young) appeared to facilitate the transition from PY to YAF, perhaps because they had resources to fall back on in less than optimal conditions. This is a concept followed by some wildlife carers as well in hand rearing wildlife by releasing them in slightly advanced body condition, so the timing of establishing their own territories and finding food, water or shelter is less critical (Gates, pers.comm.).

The mean testis weight of adult study animals was slightly higher compared to results listed by Rose *et al.* (1997). Older fostered bettong young had a lower mean testis weight than younger fostered bettongs in late lactation and subsequently produced offspring later at the age of one year or older. Rose (1989)

stated that Tasmanian bettongs reach maturity at the age of nine months. Sadleir (1969) described the effects of ecological factors such as nutrition on the onset of puberty in eutherian and marsupial species and stated that a lack in nutrition can cause a delay in puberty. The older foster bettongs showed such a delay, which was probably due to the inappropriate milk composition provided by the transfer mother.

Even though only a limited amount of growth data for hand-reared animal was available for analysis as part of this thesis, hand-rearing appeared to be a possible management tool for rearing transferred young. It is a technique widely practiced in zoos, sanctuaries and by the general public trained as wildlife carers (Taggart *et al.* 1997). Its contribution to the scientific community has been questioned on many occasions and researchers have advised against its use due to resulting behavioural problems (Brambell & Jones 1977). However, valuable information gained on artificial rearing techniques; milk formulae; identification, intervention and whenever possible prevention of disease (gathered on a species level) should not be ignored.

A major advantage of cross-fostering is that the procedure can be conducted in the very early stage of pouch life, when the chances for successful hand-rearing are close to zero. Hand-rearing has proven to be a good 'back up' solution in the event of rejection, but could also be applied for young with severely retarded growth. This opportunity has to be considered when using TAD as a management tool, since younger animals transferred into more advanced pouches will most likely show accelerated growth, but might have to leave the pouch earlier than normal for their age. Substitute feeding (Johnson 1981) and hand-rearing have to be available to satisfy the young's needs. Although it is a time and labour intensive technique, its possible benefit should not be overlooked.

Species-specific needs have to be considered to avoid over-feeding when the young is not able to control the milk intake, which can lead to obesity or even death. Growing echidna young, for example, suckle infrequently in the wild and are left in the den up to 10 days – providing the hand-reared young with milk on

demand on a more frequent basis would most likely have lethal consequences for the young (Gates, pers.comm.). Robbins (1983) reviewed hand-rearing for a variety of species, including marsupials, and pointed out the inappropriateness of over- and under-feeding young. Young do not appear to control milk intake in an artificial environment (this study). The natural clues, required by the young to sense its 'state of fill', are missing (Robbins 1983). It is therefore important to understand the entire lactation cycle to provide hand-reared young with an appropriate milk composition and amount. This also applies to the cross-fostering approach by carefully selecting the appropriate mother species.

Chapter 6: Behaviour

6.1 Introduction

Knowledge of the normal range of behaviour in a species is a useful tool in managing captive colonies, since it can be used as a measure of individual performance as well as an indicator of animal health, if changes in the behaviour patterns can be detected in time (Koontz & Roush 1996). The comparison of behaviour in captive and wild animals is often used as a welfare indicator, but Veasey *et al.* (1996) pointed out that non-performance of some wild-type behaviour patterns might be due to many behaviours being stimulus driven rather than internally generated. It is a challenge but also a necessity for captive breeding programs to provide animals with adequate environmental enrichment to encourage more behavioural options, which in turn will allow the animal to cope better with stressful events in its environment as well as alleviate boredom (Carlstead 1996).

Thompson (1996) referred to the period of the young's dependence on its mother for nourishment and protection as "buffered from the demands of the adult world" and suggested that it is therefore an opportunity for protected growth and learning.

Various aspects of mother-young interactions have been investigated in eutherian as well as marsupial species, e.g. behaviour associated with key events in the mother-young relation ship (birth, parturition, pouch life, if applicable, to weaning and beyond depending on social structure) (for example Arman 1974, Russell 1982, Renfree *et al.* 1989, Russell 1989), behavioural elements on the species-specific level (Stodart 1966, Russell 1973, Pratt 1979, Russell 1984, Johnson 1987, Virtue 1987, summarized for the Macropodoidea in Coulson 1989, Nowak *et al.* 2000), vocal communication (Happold 1972, Baker & Croft 1993), kin recognition (Todrank & Heth 2001), play behaviour (Lissowsky 1996, Byers 1999) and parental investment (Johnson 1986, Stuart-Dick & Higginbottom 1989, Ashworth 1996).

Behavioural aspects of pouch young transfers have been investigated less intensely. Merchant and Sharman (1966) found that the transfer mothers accepted all young regardless of species differences for at least a short period of growth. They needed to remove one young due to over-grooming being exhibited by the transfer mother. They also reported that transfer mothers responded to calls made by the transfer young. Johnson (1981) observed rejection of older transfer pouch young regardless of exhibited 'following' behaviour and distress calls.

The aim of this study was to determine if inter-species transfer has an impact on the young's ability to exhibit species-specific behaviour patterns as well as on successful mate recognition, reproduction and subsequent rearing of offspring (in matured transfer females).

6.2 Methods

6.2.1 Visual Animal Identification

A visual form of identification was needed for identifying individuals on video-footage. Different approaches were tested for providing the animals with distinct markers identifiable from video-recordings. Neck collars were trialed, which displayed different patterns rather than colours, since the nocturnal behaviour of the animals was recorded with a grey-scale camera (see 6.2.2.2 Technical Environment). This approach was abandoned for several reasons. Firstly, the collar disappeared from view in the neck fur requiring substantial fur removal to allow collar pattern visibility. Secondly, animals displayed signs of discomfort in wearing the collars (for example abrasion around neck and repeated attempts of removal), although they were leather made and contained a piece of elastic material for allowing comfort and growth if fitted on offspring. In the case where potoroos were housed in a group, the collar was chewed off by another group member.

Fur dying was rejected due to animal welfare concerns. The avoidance of skin contact with the involved chemicals could not be guaranteed. A distinct pattern would have only been achievable by restraining the animal for at least 30 min-

utes and avoiding contact of any other material with the applied chemicals, which could blur the intended marking. Since this type of procedure was considered to be very stressful for the animal, chemical application and removal would have required extended periods under anaesthesia, not regarded as acceptable for this study.

The final decision was made in favour of fur clipping. This technique was easily and quickly done and did not introduce any new substances that animals could otherwise remove or interact with. It used the darker colour of the under coat, which was exposed when removing the guard hair. General shapes were assigned to identify the mother (vertical line) and young (circle on neck) as well as a male (vertical line) if present. These shapes were cut into the fur on the back of the animal, since this part of the body was mostly visible on the recorded footage due to the view angle of the camera (6.2.2.2 Technical Environment). These markings were semi-permanent and needed to be maintained depending on rate of fur re-growth. Wherever possible, this maintenance was carried out during other data collection procedures to avoid unnecessary exposure to stress as a result of repeated capture.

6.2.2 Cage design

6.2.2.1 Captive Environment

The design of the video cages reflected the compromise of providing the animal with environmental enrichment while keeping it visible for the camera most of the time. All cages had a natural soil floor with a corrugated iron roofed shelter covering a quarter of the surface area. A wooden nest box and a variety of different nesting materials were offered underneath (hay, straw, gum bark, fresh grass). A piece of shade cloth hung from the roof covering the nesting area and providing privacy and sun protection during the hot summer months. A variety of native plants (Appendix A.1 Native plants) were added to the existing grass cover, positioned around a wooden log, which was situated in the middle of each cage. Environmental enrichment was provided on a continuous level (2.2.5 Enclosure types and environmental enrichment).

Grasses were continuously trimmed and/or replaced. Trees were pruned to not outgrow the cages. For each individual tree the branches were bound together when recording at night, facilitating an almost unblocked view of the cage floor. The cages were built on a slope, resulting in a marked soil moisture gradient. Bettongs were housed in the drier top row of cages while potoroos occupied the middle and lower row, resembling the moister conditions of their natural habitat. Potoroos were also provided with half pipe plastic tunnels since they seemed to prefer nesting sites with two openings, possibly for better ventilation or escape. Visual contact between different species was avoided by the alignment of the cages as well as the use of shade cloth on cage walls, with the exception of cross-foster mother-young dyads.

The importance of the cage design lay in the identical positioning of shelter, logs and plants as well as technical facilities in each video-cage, providing similar conditions for all housed mother-young-dyads to acclimatise to. This approach maximised the number of animals for the recording of behaviour while minimising the stress they would have been exposed to in a 'one cage' set-up with fixed equipment (for example frequent handling, constant change to acclimatise to). Each morning the technical equipment was transferred into the appropriate cage for the following nights recording with minimal disturbance to the animals in their nests.

6.2.2.2 Technical Environment

The amount of cables accessible to the animals within the cage was kept to a minimum to prevent potential injury. Cables were enclosed in plastic tubing to discourage chewing behaviour. Wherever possible cables were attached to the wire ceiling rather than the cage walls, since both bettongs and potoroos are excellent climbers.

The audio-visual equipment used within the cage consisted of a weatherproof grey-scale bullet camera (X POSE, Cat. QC-3464, Appendix A.7 Camera specifications) and an electrolytic omnidirectional microphone with a pre-amplifier

(Jaycar Electronics, Australia). Both were powered by a regulated 12V power source. The camera was attached to a metal bracket located on the wooden doorframe of each cage opposite to the shelter area and close to the ceiling. A digital camera (SONY Digital Handycam, DCR-TRV11E) was used as a mobile monitor for accurately aligning the bullet camera. The height of the camera allowed the 92°-angle view to cover the entire cage floor with the exception of most of the shelter area in some of the cages. The microphone was placed 50cm above ground level, facing the cage. It was positioned on the outside to discourage investigation by the study animals.

A 'string of lights' was used as a light source during night-time recordings, consisting of a 240V standard wiring cable with ten light globe sockets. Red light globes (40W) were used, which provided sufficient light for the camera. Both positioning and use of the appropriate number of light globes were employed to vary lighting conditions according to the individual cage properties (e.g. ceiling height). The string of lights was connected to an automatic timer, which turned the power on at dusk to ensure a smooth day-night transition for the animals to be recorded. It also turned the power off at the end of the recording.

Camera and microphone were connected via coaxial cable to a balan situated near the animal enclosures. From there both audio and video signal were transmitted across a ~250m link consisting of Cat-5 cable to a second balan located in the Zoology department. Another set of coaxial cables led both signals to the VCR (Panasonic Time Lapse Video Cassette Recorder, Model: AG-6730E) for recording. This was connected to a monitor, which was used the following morning to verify the quality of the gathered footage.

6.2.3 Video-recording

6.2.3.1 Recording Regime

A female was placed in a video-cage while her young was still in the pouch. She was usually allowed two weeks to acclimatise to the new environment before recordings commenced. Since several mother-young dyads occupied the

video-cages, each one had to be recorded in turns. The behaviour of mother and young was recorded for the period from permanent pouch emergence through to weaning. Recordings of 12 hours length were conducted at the beginning and end of this phase. Since the activity phase of potoroos exceeded 12 hours, 24 hours recordings were conducted accordingly. Weekly recordings of three hours length were obtained for the above mentioned period for each mother-young dyad. Dusk was chosen as the starting time (between 5 and 9pm depending on time of year and weather conditions) to cover the beginning of the animals' activity phase.

Great care was taken to avoid human interference during recording. The mother-young dyad was excluded from data collection (for example milk sample collection or growth measurements) on the day of recording whenever possible. Students and staff left the enclosure approximately 30 minutes prior to recording. Automatic timers were employed for operating lights and recording equipment.

6.2.3.2 Digitisation

All videotapes (VHS) were digitised into AVI media files due to the generation of data transfer errors between the VCR and the Observer software (e.g. time code and image quality). The video signal, which was captured on tape, had incorrect voltages on it, causing the resultant image to be so bright as to be saturated in places. The unnatural video signal was corrected by connecting a variable resistor between the video out wire and ground.

The software 'iuVCR' (version 4.8.5.331, iuLab) was used for digitising the collected video data, utilising a video capture card (WinFast TV 2000 XP Expert WDM Video Capture, installed in P4, 2.8GHz, 1GB RAM). A series of test trials led to the development of settings for capture format (frame size: width 720/height 576, frame rate: 25fps) and audio format (MPEG Layer-3, 64kBit/s, 48.000Hz, Mono, 7 Kb/sec). The DivX Pro ™ 5.1.1 Codec was used for compression, de-interlacing all frames.

The lighting conditions changed during a recording session due to a starting time at dusk. Therefore the tape was positioned one hour into the recording for adjustments. A multi-system time base corrector (model no. CTB-100) in conjunction with a histogram (iuLab Histogram/iuVCR filter) was utilised to alter image brightness and contrast whilst maximising the input for the capture card. The histogram represented these adjustments as a bell shaped curve spreading across the graph. The iuVCR channel settings for brightness and contrast finalised the modifications by removing any signal abnormalities, extending the curve even further. After rewinding the tape the recording process was started. An Alpary denoise filter (iuVCR filter) was applied for removing video noise while digitising the videotape data in real time. The resulting AVI file had a size of approximately 2.3GB and was accessed for analysis from the hard drive. It was scanned using the software VirtualDub (version 1.5.7) to verify the quality of the digital information and facilitate data selection for subsequent analysis. After completing the analysis two files were burned onto a DVD+R for storage purposes.

6.2.4 Behavioural Analysis

6.2.4.1 Data selection

Animals

Only mother-young dyads of both species with original young and cross-fostered young were chosen for the behavioural analysis due to time constraints. Therefore dyads with fostered young as well as fostered or cross-fostered young as adults rearing their offspring were excluded from analysis for this project. Exclusion also applied to dyads with young dying at some stage of the recording process. Mothers with disabilities (e.g. deafness or loss of an eye) were not included in the analysis. Although having reared their young successfully, they had to alter their behaviour in order to compensate for the impediment.

Development of young

In accordance with the restrictions outlined above, collected video data was considered for analysis from when the young left the pouch permanently until

weaning. The interactions of mother and PY were not included, since most of this activity occurred in the nest which was not accessible by the camera. Virtue (1987) detected major changes in the relationship between bettong mother and young occurring within the first 40 days after permanent emergence from the pouch. The weekly collected data covering the described period was analysed for the appropriate mother-young dyads in this study (see 6.2.4.2 Configuration).

'Tape position'

A 30 minutes section of the collected data was analysed for each mother-young dyad per week. The starting point of analysis was laid down for all recordings by the event of both mother and young initially appearing in sight together independent of the actual time of night. This procedure was necessary since some animals chose to remain in their nests for prolonged periods. The second analysis requirement consisted of both mother and young remaining in sight for at least 20 minutes of the chosen observation time. If this requirement could not be fulfilled in accordance with the appropriate analysis starting point as described above, another 30 minutes segment was selected from the same media file. If no such segment was found, the longest duration of mother and young in sight was chosen to gain a better understanding of their behaviour, but was excluded from the statistical analysis.

6.2.4.2 Configuration

The software package "The Observer" (version 4.1.126, Noldus Information Technologies) was used for analysing the recorded behavioural data. A single actor configuration was written for analysing the behaviour of mother and young as main actor in turns (Appendix A.8 Configuration review). The duration of the observation to be analysed was 30 minutes with the observation timing based on elapsed time. A total of eight independent variables were assigned, including focal animal ID, species (bettong/potoroo), sex (male/female), category (mother/young), pouch week (13 – 36 weeks), age (13 – 36 weeks or adult), transfer type (original/foster/cross-foster) and age difference at time of transfer

(same age/younger/older). Since animals were housed in different grouping arrangements (2.2.6 Housing), all other present individuals were listed as possible subjects for interactions, including other young, other group member, neighbour and vermin.

"The Observer" is structured in a similar way to an ethogram, dividing the behaviour into distinct categories and describing it to the most detailed level. A behavioural class consists of behavioural elements, which can be classed as states or events. Depending on the required level of detail, two modifiers can be assigned for each element. Behaviour of different classes can be displayed simultaneously.

The main focus for this analysis was to determine species-specific behaviour and the process of the young gaining independence from its mother. For this approach individual and social behaviour of mother and young, and the distance between them were recorded in separate behavioural classes. Individual behaviour was described with the following behavioural elements: resting, locomotion, vigilance, feeding, drinking water, investigation, auto-grooming, over-balancing and other individual behaviour. All behavioural elements were classed as states with the exception of over-balancing as an event.

For social behaviours, the elements approaching, distancing, following, sniffing, copying, taking food away, approach other subject, distance other subject and follow other subject were classified as events. The elements social encounter, allo-grooming, pouch related behaviour, interaction with other subject, agonistic and sexual behaviour were categorised as states. The elements 'other individual behaviour' or 'social encounter' were used when the observed behaviour could not be classed in any other individual or social element category or if the view was obstructed and only the class not the element could be determined. It was also noted when the main subject was out of sight (a state element).

The use of modifiers helped to place behavioural elements into a more detailed context. As with the behaviour, modifier elements are organised in modifier classes. Six modifier classes were allocated for this analysis. The class 'kind' was assigned to give a better insight into the nature of interactions with others, which could be classified as social, agonistic, avoiding or sexual. The behavioural element 'locomotion' was described in more detail by the modifier class 'type', giving the options of biped, quadruped, stereotypic, foray, climbing or locomotion other. Forays are defined as fast biped hopping excursions, circling around or leading from and towards the mother. Investigation behaviour was divided into digging, nest building and other investigation behaviour by usage of the modifier 'form'.

Since agonistic and sexual behaviour between mother and young was observed on rare occasions, appropriate modifier classes were allocated. Agonistic modifier elements included threatening, attacking, avoiding, defending and other agonistic activity. For the sexual modifier class the elements investigation, mating attempt, copulation and other sexual activity were chosen. When an animal was out of sight, modifiers were assigned to give additional information about its location (for example in the nest, shelter or other location). The most efficient way of gaining in-depth information about a particular behaviour was by combining two modifiers. This was the case for the behavioural element 'interaction with others' with modifier 1 ('subjects') explaining who participated in the interaction, while modifier 2 ('kind') gave an insight in the nature of the interaction.

Great care was taken to determine the appropriate duration of a behavioural state. When state behaviours obtained an event character, a clear distinction was made between interrupting and alternating states. For example, if an animal exhibited investigation behaviour, a little movement to the side was a form of locomotion, another behavioural state in the same behavioural class, which cannot be displayed simultaneously. Since the investigation behaviour was continued after moving to the side, the main behavioural element remained investigation and the record of locomotion was omitted. If the movement turned

into a pronounced way of locomotion or led to a different behavioural element, it was recorded as a new behavioural element, terminating the preceding investigation behaviour.

The behavioural class 'distance' consisted of eight elements (body contact, up to 10cm, up to 50cm, up to 1m, up to 2m, up to 3m, up to 4m and distance unclear) with the distinction of mother and/or young utilising the open space of the cage or seeking protection (e.g. shelter or nest). All elements were classed as states. Distance data was recorded as a continuum and only analysed for the mother, since it remained the same independent of which subject's perspective was considered. If mother or young were out of sight but their location was known, the recording of the distance between them was continued. If the location was not known or both were out of sight (with the exception of being in the nest), the category 'distance unclear' was chosen.

Hinde and Spencer-Booth (1968) used a coefficient for change in distance between mother (M) and young (Y) to determine which animal is responsible for the maintenance of proximity. It is based on the frequencies of the behaviour patterns 'approaching' (A) and 'distancing' (D) of both actors and calculated using the following equation:

$$\text{approach-distance-coefficient} = \left(\frac{A_Y}{A_Y + A_M} \times 100\right) - \left(\frac{D_Y}{D_Y + D_M} \times 100\right)$$

Positive coefficient values indicate that the young is maintaining the proximity to the mother, while negative values specify the mother as the initiator. In this study the results for the behaviour pattern 'following' were included in 'approaching' since both were interpreted as an attempt to maintain proximity between mother and young.

6.2.4.3 Statistical Analysis

A One-Way-ANOVA with subsequent *post-hoc* comparison (Least Significant Difference t-Test, 0.05 level of probability) was performed to detect significant

differences between untransferred (original) and cross-foster actors (mother and young of both species) for behaviour patterns and distance categories. Tests were conducted for each of the six observation weeks separately (2.2.13 Data management and statistical analysis). Observations were excluded from analysis if the actor was out of sight for more than ten minutes of the total observation time. When calculations were based on the results of mother and young (e.g. distance, 'approach-distance-coefficient') observations of both were excluded from analysis if one or both actors were out of sight for more than ten minutes.

6.3 Results

The behaviour of mother-young pairs was recorded for 420 nights between November 2000 and May 2002 for a variety of mother-young combinations (untransferred, fostered, cross-fostered as well as mature transfer animals as parents). A total of 132 observations from 22 animals were included for the behaviour analysis according to the selection criteria described under 6.2.4.1 Data selection. The dataset is derived from three individuals per actor (mother/ young), species (bettong/potoroo) and transfer category (cross-foster/original) with the exception of only two potoroo mothers and their cross-foster bettong young over the six week period following pouch vacation. In the graph description 'N' is provided as the number of actors or pairs/category.

Data of both actors (mother and young) for each species are presented together to facilitate easier cross-species comparisons. It also assists in comparing the behaviour of the young with the final stage of mother behaviour, which is used as a guideline in assessing the progress of the young's independence establishing with time. Sudden changes in the behaviour are best not overrated, since the behaviour analysis was restricted to a very small number of animals. These changes most likely represent individual changes rather than general trends.

6.3.1 Individual behaviour

The behavioural class 'individual behaviour' (Fig.6.1) combined the elements 'vigilance', 'investigation', 'locomotion', 'feeding', 'auto-grooming' and 'other individual behaviour'. Most of these elements are described subsequently, since they were exhibited for the majority of analysis time.

Original bettong mothers displayed less individual behaviour with time. The mean total duration for individual behaviour in week 1 after the young's permanent emergence from the pouch (PEP) decreased from 95% to 89% in week 6. Cross-foster bettong mothers on the other hand exhibited individual behaviour almost at all times with values ranging from 97% to 99%. This resulted in a significant difference in mean total duration of individual behaviour between the two bettong mother categories in week 3 [$F_{(1, 4)}$=12.012, p=0.026].

On the contrary, the results for original potoroo mothers showed an increase of time spent with individual behaviour. The mean values rose from 79% to 90% over the six week period after PEP. Results for cross-foster potoroo mothers remained stable at ca. 80% for the first three weeks, increased to 92% in the following week and subsequently decreased to 51% at the end of the analysis period.

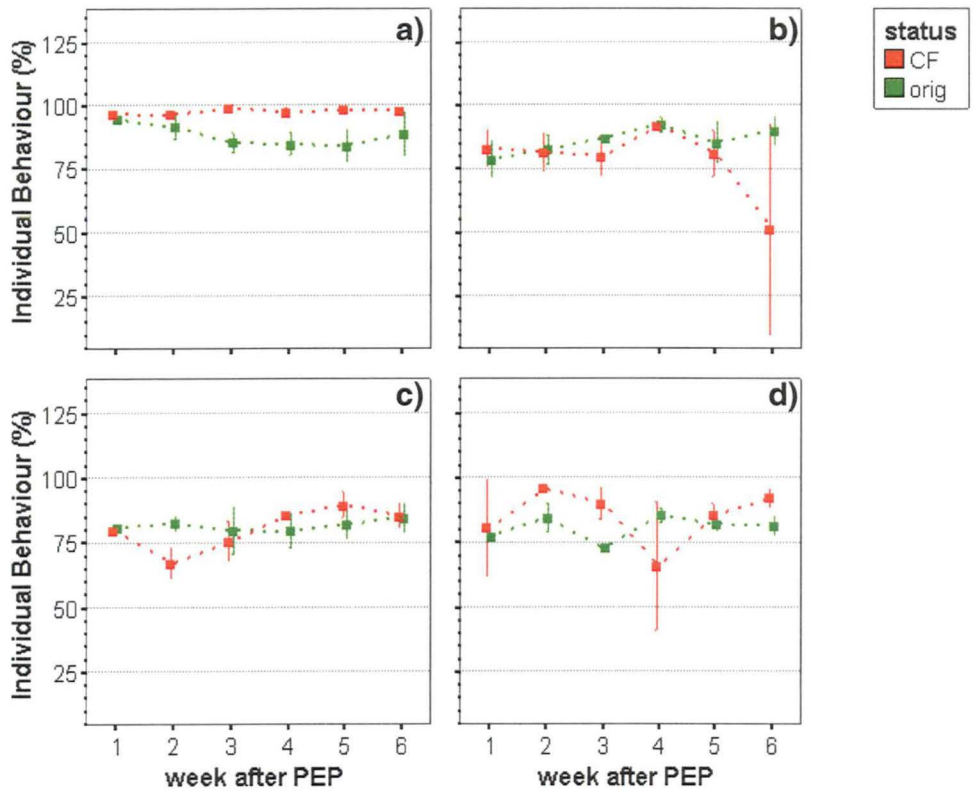

Fig.6.1: Changes in total duration (% of observation) of individual behaviour exhibited by bettong mothers (a, N=3/CF, N=3/orig), bettong young (c, N=2/CF, N=3/orig), potoroo mothers (b, N=2/CF, N=3/orig) and potoroo young (d, N=3/CF, N=3/orig) for the six week period following permanent emergence from the pouch (PEP). The status of the actor is displayed in red for cross-foster and green for original animals. Error bars show Mean ± 1.0 SE.

The values for individual behaviour shown by original bettong young remained mainly constant just above 80% throughout the entire analysis period with a slight increase to 85% in week 6. Values for cross-foster bettong young initially decreased from 80% to 67% in week 2, but subsequently rose to 85% of observation time in the final week. The results of both original and cross-foster bettong young for week 6 corresponded with the value obtained for original bettong mothers.

Results for both original and cross-foster potoroo young increased during the analysis period with varying degrees of fluctuation. The amount of time spent with individual behaviour rose for original potoroos from 77% to 82% in the six week period. Results for cross-foster potoroos were slightly higher, increasing

from 81% to 92% of observation time. While the final result for original potoroo young in comparison with adult potoroos appeared to be too low, the value for cross-foster young corresponded with the adult results for week 6.

6.3.1.1 Vigilance

Values for vigilant behaviour (Fig.6.2) of both bettong mother categories decreased over the analysed period from 53% to 39% for cross-foster mothers and 42% to 33% for original mothers respectively.

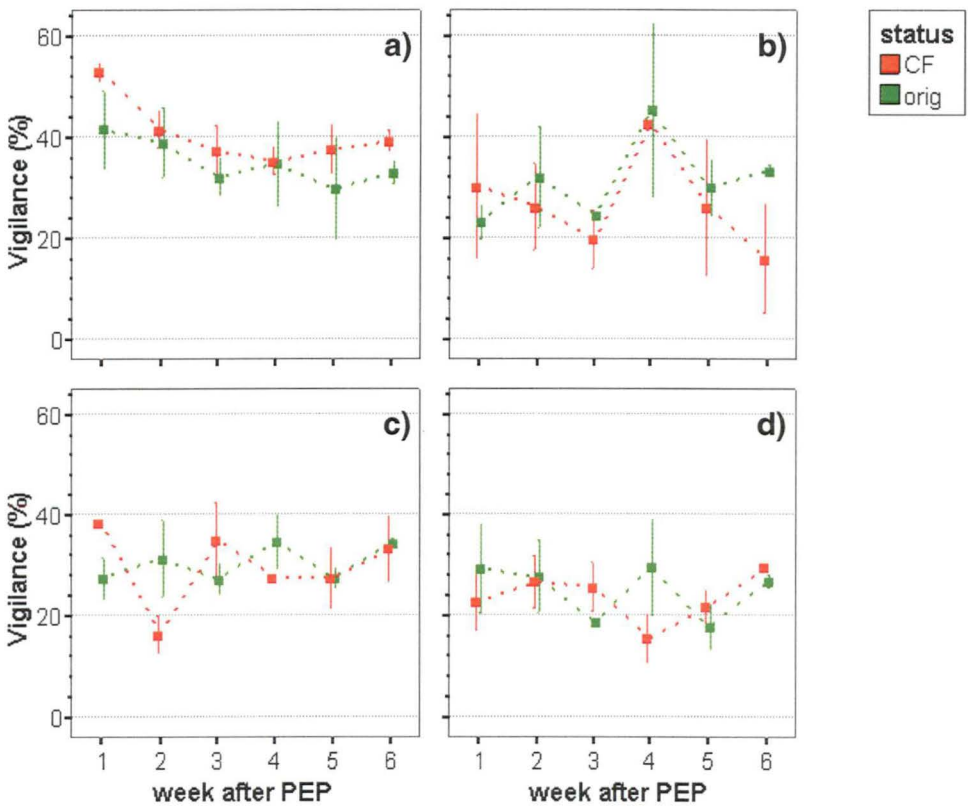

Fig.6.2: Changes in total duration (% of observation) of vigilance exhibited by bettong mothers (a, N=3/CF, N=3/orig), bettong young (c, N=2/CF, N=3/orig), potoroo mothers (b, N=2/CF, N=3/orig) and potoroo young (d, N=3/CF, N=3/orig) for the six week period following permanent emergence from the pouch (PEP). The status of the actor is displayed in red for cross-foster and green for original animals. Error bars show Mean ± 1.0 SE.

The mean total duration of vigilant behaviour displayed by potoroo mothers was slightly lower (week 1: orig = 23%, CF = 30%). Values increased over time for original potoroo mothers (week 6: 33%) and decreased for cross-foster potoroo

mothers (week 6: 16%). Mean values for original bettong young increased slightly from 27% to 34% over the six week period with good correspondence of the final results between original bettong mother and young. The decrease of values for vigilance in the mothers compared to the increase in vigilant behaviour in the young suggests a growing independence of the young with time. Results for cross-foster bettong young were initially higher (week 1: 38%) and underwent greater fluctuations before reaching 33% in the final week, which is adequate in comparison with adult females of both species.

Mean values for total duration of vigilant behaviour in original and cross-foster potoroo young were slightly lower and varied between ca. 20% and 30% of observation time. There was a slight increase of vigilance in cross-foster potoroo young over time from 23% to 29%, while results for original potoroo young remained mostly constant (week 1: 29%, week 6: 27%). The final values of both potoroo young categories corresponded with the results for original adult potoroos.

6.3.1.2 Investigation

This behavioural element combined manipulative behaviour and various forms of investigation including the occurrences of digging and gathering of nesting material (Fig.6.3). Original bettong mothers spent about 24% of the observation time investigating their environment throughout the six week analysis period. Cross-foster bettong mothers showed an increase in mean total duration of investigation behaviour from 17% to 27% in the first two weeks after the young left the pouch. Values remained between 27% and 23% over the following three weeks and subsequently decreased to 13% in week 6. The mean total duration of investigation in original potoroo mothers remained constant at 12% for most of the analysis period, while values for cross-foster potoroo mothers declined steadily from 19% to 5% over time. This resulted in a significant difference between the two potoroo mother categories in week 3 [$F_{(1,\ 1)}$=337.080, p=0.035].

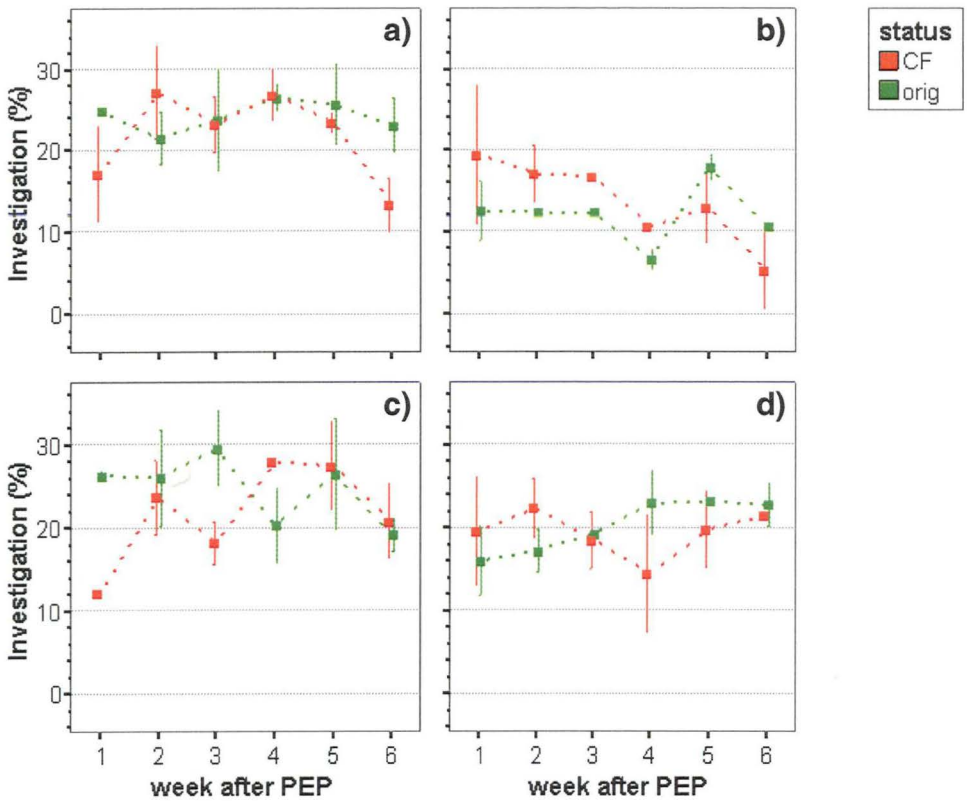

Fig.6.3: Changes in total duration (% of observation) of investigation behaviour exhibited by bettong mothers (a, N=3/CF, N=3/orig), bettong young (c, N=2/CF, N=3/orig), potoroo mothers (b, N=2/CF, N=3/orig) and potoroo young (d, N=3/CF, N=3/orig) for the six week period following permanent emergence from the pouch (PEP). The status of the actor is displayed in red for cross-foster and green for original animals. Error bars show Mean ± 1.0 SE.

The values for both bettong young categories showed contrary trends. The results for mean total investigation duration decreased for original young (26% to 19%) and increased for cross-foster young (12% to 21%) with time. A significant difference between the two bettong young categories was detected for week 1 [$F_{(1, 1)}$=269.695, p=0.039]. Mean values rose for original potoroo young from 16% to 23% over the six week period following pouch vacation, but oscillated around 19% for cross-foster potoroo young. The final results for mean total duration of investigation for young of both species ranged from 19% to 23%, which corresponded with the data of adult original bettong mothers, but exceeded adult potoroo results.

6.3.1.3 Locomotion

This behavioural element consisted of various forms of locomotion such as biped and quadruped movements, forays, climbing and other types including stretching, when the body was lengthened and partly dragged across the ground (Fig.6.4).

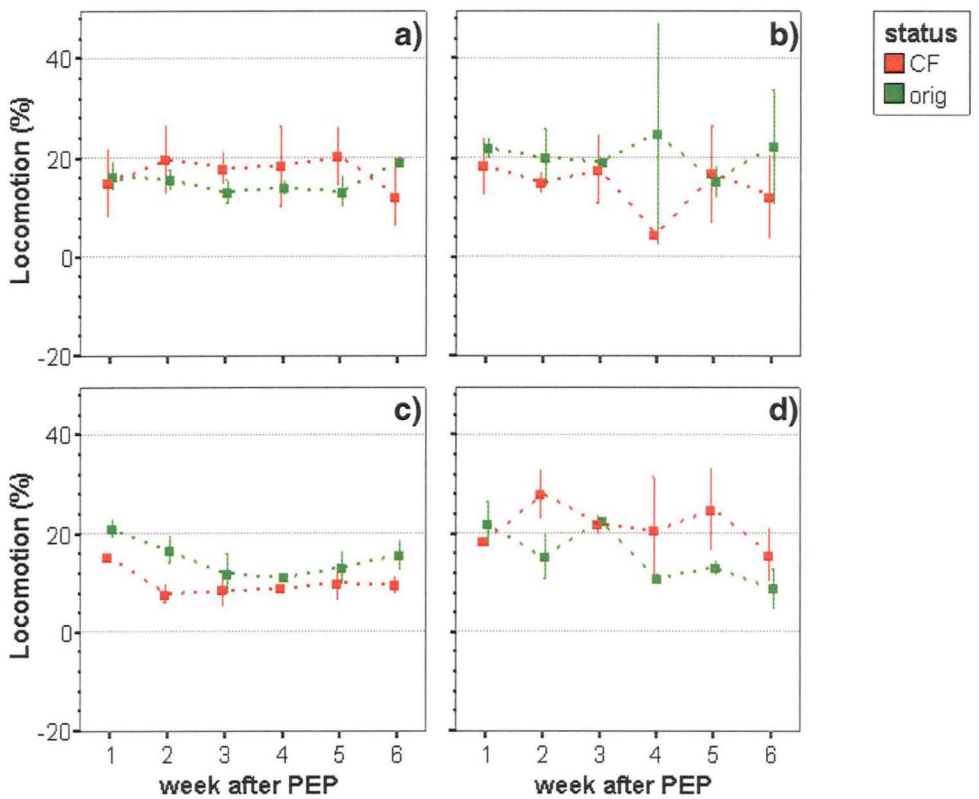

Fig.6.4: Changes in total duration (% of observation) of locomotion exhibited by bettong mothers (a, N=3/CF, N=3/orig), bettong young (c, N=2/CF, N=3/orig), potoroo mothers (b, N=2/CF, N=3/orig) and potoroo young (d, N=3/CF, N=3/orig) for the six week period following permanent emergence from the pouch (PEP). The status of the actor is displayed in red for cross-foster and green for original animals. Error bars show Mean ± 1.0 SE.

The total duration of locomotion in original bettong mothers remained constant at ca. 15% throughout most of the analysis period with an increase to 19% in the final week. Initial values for cross-foster bettong mothers were similar, but stayed at ca. 19% between week 2 and 5 and subsequently decreased to 12% in week 6. Locomotion results for original potoroo mothers remained mostly at a mean total duration of just above 20% of the observation time throughout the analysis period and 14% respectively for cross-foster potoroo mothers.

Results for bettong young showed a slight decrease in time spent on locomotion. Values for original bettong young fell from 21% to 16% over time with a low of 11% in week 4. Results for cross-foster bettong young decrease in the first two weeks from 15% to 8% and remained constant thereafter. Original potoroo young spent less time on locomotion towards the end of the analysis period with initial levels of 22% decreasing to 9%. The final mean total locomotion duration, however, appeared to be too low when compared to adult potoroo results.

Cross-foster potoroo young followed a similar trend compared to their cross-foster mothers. Results for mean total duration of locomotion increased from 19% to 28% in the first two weeks, remained at ca. 24% until week 5 and subsequently decreased to 16% in the final week. Final values for mean total locomotion duration for young of both species are too low compared to their original adult counterparts.

Bettong mothers favored the biped form with a mean frequency for cross-foster mothers rising from 110 to 139 events in the first two weeks, followed by a second increase to 151 events in week 5 with a subsequent decrease to 86 events per observation in the final week (Fig.6.5). The mean frequency for original bettong mothers remained constant at ca. 90 events per observation throughout the analysis period with an increase to 123 events in the final week. Potoroo mothers rarely exhibited biped locomotion. Mean frequencies ranged from 2 to 9 events for cross-foster potoroo mothers and from 1 to 5 events for original potoroo mothers respectively.

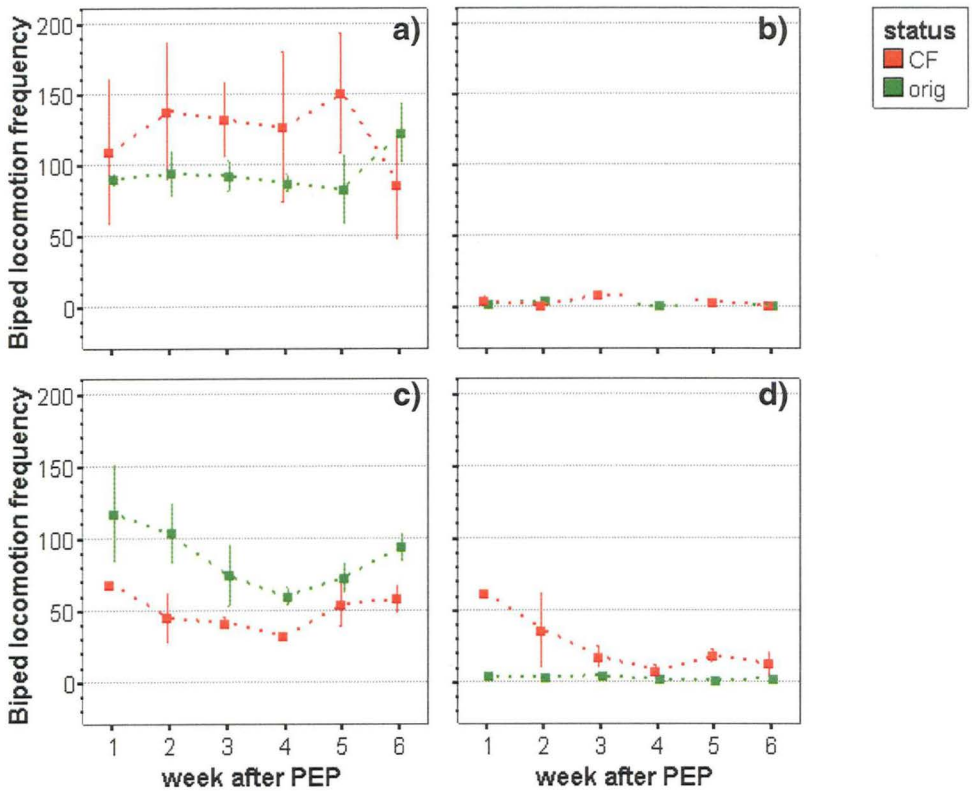

Fig.6.5: Changes in frequency (mean number of events per observation) of biped locomotion exhibited by bettong mothers (a, N=3/CF, N=3/orig), bettong young (c, N=2/CF, N=3/orig), potoroo mothers (b, N=2/CF, N=3/orig) and potoroo young (d, N=3/CF, N=3/orig) for the six week period following permanent emergence from the pouch (PEP). The status of the actor is displayed in red for cross-foster and green for original animals. Error bars show Mean ± 1.0 SE.

The mean biped locomotion frequency for original bettong young was halved by week 4 (decreasing from 118 to 60 events per observation). The result of 94 events per observation in the final analysis week was comparable with results from adult bettongs. Cross-foster bettongs, however, produced much lower results with a decrease in mean frequency from 69 to 33 events per observation in the first four weeks. In the final observation week a mean of 59 events of biped locomotion was recorded for cross-foster bettongs – a value, which exceeded the results of the cross-foster mothers, but also failed to meet the standard set by bettong mothers.

The same trends could be seen for potoroo young with results from original young ranging from 2 and 5 events per observation over time. The frequency for cross-foster potoroo young on the other hand produced a peak of 62 events per observation in week 1, which was significantly different from original potoroo young [$F_{(1, 1)}$=4408.333, p=0.010]. Results subsequently decreased with time and remained at a rate of ca. 15 events per observation over week 3 to 6.

The quadruped type was the preferred form of locomotion for the potoroo mothers. The mean frequencies for both original and cross-foster potoroo mothers described a decreasing trend. Mean values fell from 188 to 147 events for original mothers and 174 to 97 events per observation for cross-foster mothers. The results for bettong mothers followed the same trend on a smaller scale with a decrease from 39 to 19 events for original mothers and 40 to 11 events per observation for cross-foster bettong mothers.

The results for potoroo young presented adult-like frequencies. The mean frequency for quadruped locomotion in cross-foster potoroo young was only half as high compared to original potoroo young (108 versus 205 events per observation) in the first week after pouch vacation, but adjusted to the trend of original potoroo young thereafter. Values for bettong young were slightly higher compared to adult bettong mothers, but described the same decrease over time. Mean frequency values dropped slightly from 33 to 29 events per observation over the six week period for original bettong young, while mean results for cross-foster young halved (57 to 25 events per observation) in the same time frame. This resulted in a significant difference between the two bettong young categories in week 4 [$F_{(1, 2)}$=41.441, p=0.023].

Climbing was very rarely seen in bettongs (one event for mothers and young each during six observations), while adult potoroos climbed at least between one to three times per observation. This trend was seen in the original potoroo young results as well, while cross-foster potoroo young showed an increase in climbing activity (mean maximum frequency: 11 events per observation at week 5) before reaching adult levels in the final observation stage.

Forays were mainly displayed by original and cross-foster bettong young as well as cross-foster potoroo young. Foray frequencies remained between 1 and 4 events per observation over time for cross-foster bettong young. The initial value for original young was much higher with 12 events per observation, but foray frequency subsequently decreased to a rate of ca. 2 events per observation in week 3 and 5 after pouch vacation. The mean foray frequencies for cross-foster young remained between 2 and 5 events per observation with an increase in week 4 and 5 to a maximum value of 19 events per observation. For all original potoroo young a total of one foray event was recorded in week 2 after pouch vacation.

6.3.1.4 Feeding

The feeding results for all mothers and their young (with the exception of original potoroo mothers) described an increase of time spent feeding throughout the observation period with various amounts of fluctuation (Fig.6.6). The increase of mean total feeding duration varied per category with values for original bettong mothers rising from 8% to 12% and cross-foster bettong mothers from 4% to 10% over time. Results for cross-foster potoroo mothers were situated in the same range, increasing from 5% to 10%, whereas levels for original potoroo mothers appeared to be higher and less subject to change. Except for a decline to 4% in week 4, the mean total feeding duration for original potoroo mothers remained between 10% and 12% per observation at all times.

The increase in mean feeding duration covered a slightly larger range for potoroo young (orig: 5% to 17%, CF: 2% to 19%) compared to bettong young (orig: 3% to 14%, CF: 7% to 13%), but all young produced higher results in the final observation week than their adult counterparts.

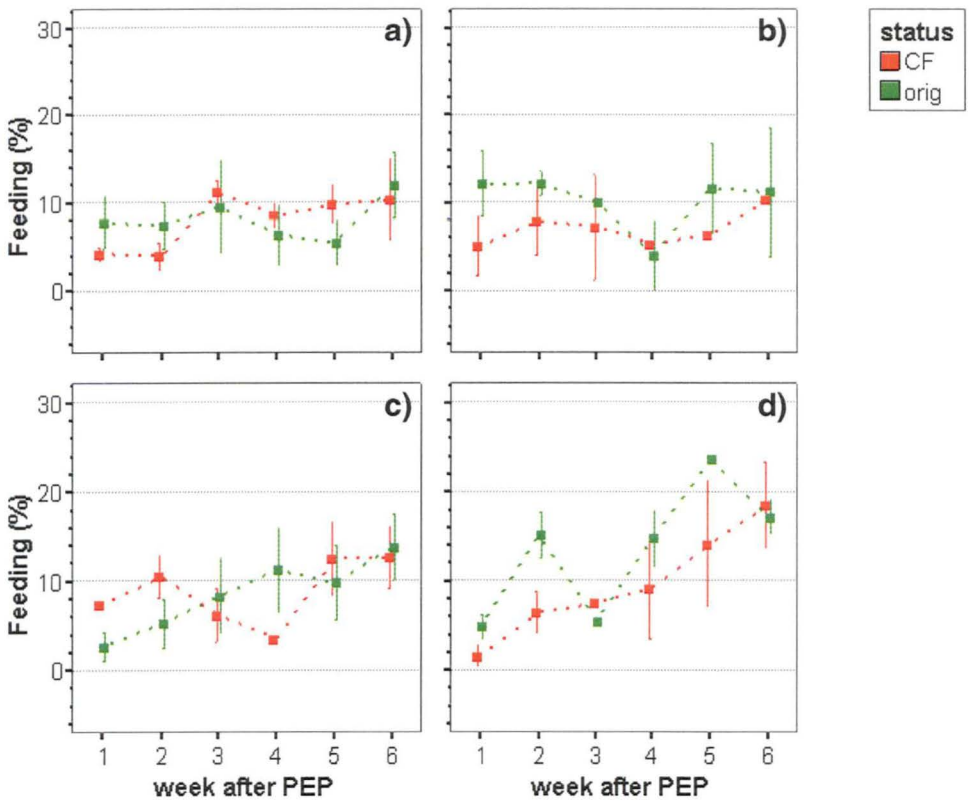

Fig.6.6: Changes in total duration (% of observation) of feeding behaviour exhibited by bettong mothers (a, N=3/CF, N=3/orig), bettong young (c, N=2/CF, N=3/orig), potoroo mothers (b, N=2/CF, N=3/orig) and potoroo young (d, N=3/CF, N=3/orig) for the six week period following permanent emergence from the pouch (PEP). The status of the actor is displayed in red for cross-foster and green for original animals. Error bars show Mean ± 1.0 SE.

The act of taking food away from the dyad partner was rarely observed (1 to 2 events per observation). It was only seen in bettong mothers (week 3 and 4) as well as in young (bettong: week 5 and 6, potoroo: week 1, 3 and 5).

6.3.1.5 Drinking

The mean drinking frequencies of young were observed for detecting possible tendencies of using water as a substitute for the decrease in milk volume provided after pouch vacation (Fig.6.7).

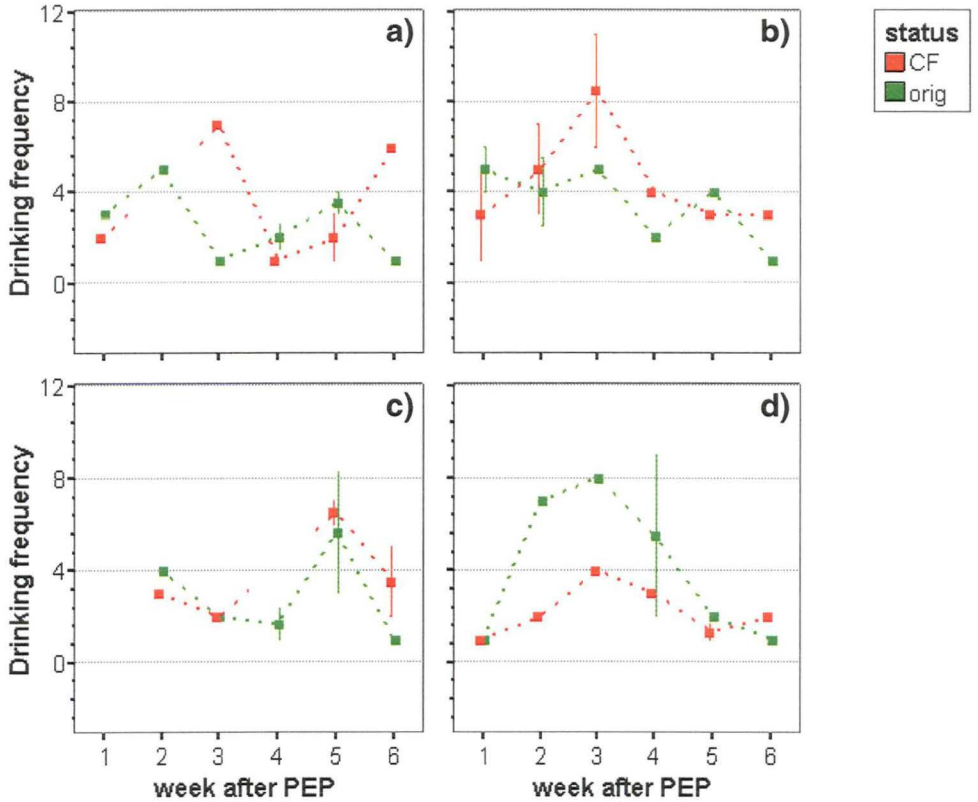

Fig.6.7: Changes in frequency (mean number of events per observation) of drinking water exhibited by bettong mothers (a, N=2/CF, N=3/orig), bettong young (c, N=2/CF, N=3/orig), potoroo mothers (b, N=2/CF, N=3/orig) and potoroo young (d, N=3/CF, N=3/orig) for the six week period following permanent emergence from the pouch (PEP). The status of the actor is displayed in red for cross-foster and green for original animals. Error bars show Mean ± 1.0 SE.

Although the mean frequency of adults drinking water showed great fluctuations over time, maximum levels were produced in the first three weeks of the analysis period. Potoroo young follow this trend with higher maximum values for original young (8 events) compared to cross-foster potoroo young (4 events per observation). Bettong young on the contrary exhibited peak drinking frequencies in the second half of the analysis period (orig: 6 events, CF: 7 events per observation). No drinking behaviour was observed at week 1 and 4 for cross-foster bettong young as well as week 1 for original bettong young. A significant difference in mean total duration of drinking behaviour was found for the two bettong mother categories in week 3 [$F_{(1,\ 2)}$=77.358, p=0.013] and week 6 [$F_{(1,\ 1)}$=363.000, p=0.033].

6.3.1.6 Auto-grooming

Auto-grooming served a hygienic purpose, but was also indicative of a stressful environment when performed excessively. Values for the mean total auto-grooming duration in bettong mothers (Fig.6.8) remained mostly constant (between 1% and 6% of the observation time) throughout the analysis period with the exception of an increase in the final week for cross-foster bettong mothers (20%). Results for potoroo mothers fluctuated slightly more (between <1% and 10%), but followed the same trend. A significant difference was found between the two potoroo mother categories in week 6 [$F_{(1, 2)}$=86.843, p=0.011]. There was an isolated peak of 28% of the observation time for cross-foster potoroo mothers in week 4. This peak was also reflected in the corresponding results for cross-foster bettong young (17%), which ranged between 4 and 8% during most of the analysis period. Values for original bettong young were slightly lower (between 1% and 3% of observation time), which resulted in a significant difference between the bettong young categories in week 5 [$F_{(1, 3)}$=17.999, p=0.024].

The mean total auto-grooming duration for cross-foster potoroo young was slightly lower during the first five weeks following pouch vacation (between <1% and 2%) compared to original potoroo young (between 2% and 6%), which resulted in a significant difference between the two young categories in week 3 [$F_{(1, 2)}$=89.286, p=0.011]. The results for cross-foster potoroo young increased in the final week of analysis, which might be a reflection of the trend seen in cross-foster bettong mothers.

Over-balancing was mostly observed in conjunction with auto-grooming behaviour of young, but was not displayed in every observation week. While only a total of six events were observed for all potoroo young (week 1, 2, 4-6), bettong young appeared to over-balance more frequently. Mean frequencies for original bettong young decreased from 8 to 1 event in the first three weeks, while cross-foster bettong young over-balanced 4 times per observation in week 1, 2 and 4. Their values subsequently decreased to 1 event in the final observation week.

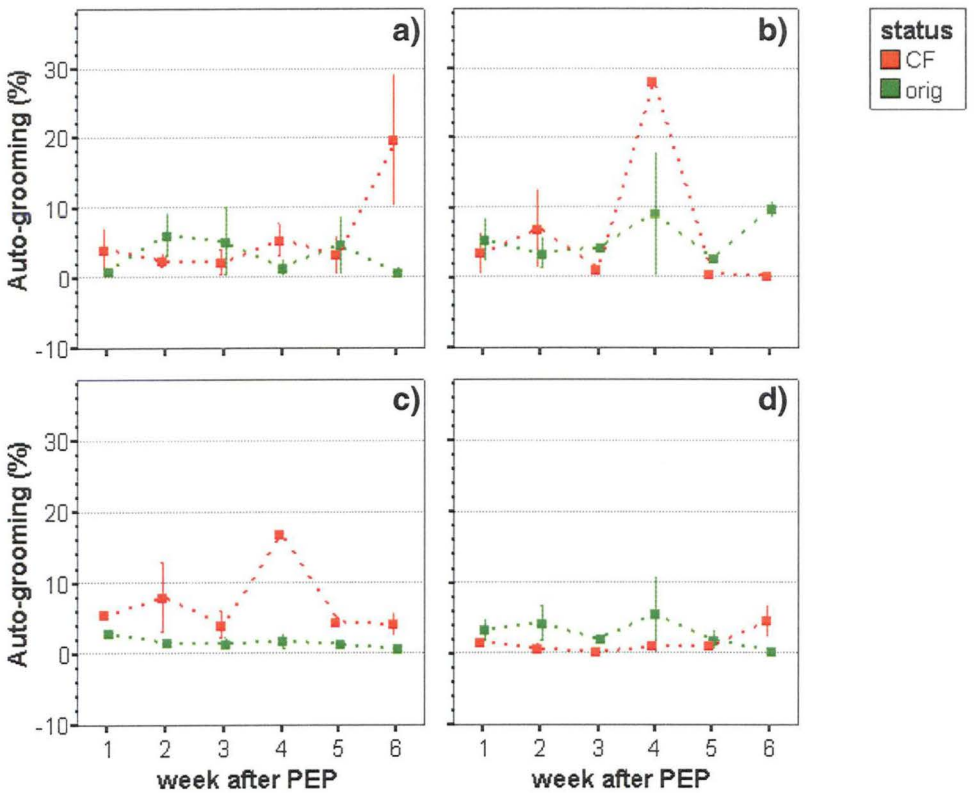

Fig.6.8: Changes in total duration (% of observation) of auto-grooming exhibited by bettong mothers (a, N=3/CF, N=3/orig), bettong young (c, N=2/CF, N=3/orig), potoroo mothers (b, N=2/CF, N=3/orig) and potoroo young (d, N=3/CF, N=3/orig) for the six week period following permanent emergence from the pouch (PEP). The status of the actor is displayed in red for cross-foster and green for original animals. Error bars show Mean ± 1.0 SE.

6.3.1.7 Nest building behaviour

Nest building behaviour has been frequently observed in the study animals. Bettongs gathered nesting material in their coiled tails and transported it to the chosen nesting site. Nests were mostly dome shaped with an opening of varying size. Potoroos mainly dragged nesting material towards them and created a mound like structure around them, which grew in size depending on availability of suitable material. Adult potoroos have only been seen on rare occasions gathering nesting material in their tails, even when excessive amounts of hay and gum bark were supplied in an attempt to stimulate nest building behaviour. Subadult cross-foster potoroo young, however, have been observed frequently gathering nesting material and on rarer occasions constructing dome-shaped

nests in a 'bettong like fashion'. Cross-foster bettong mothers have been observed to gather nesting material for over an hour at times with the cross-foster potoroo young watching her. In the behaviour data selected for analysis only one nest building event was exhibited by an original bettong young one week after permanent emergence from the pouch.

6.3.2 Social behaviour between mother and young

The class 'social behaviour' combined the behavioural state elements of 'allogrooming', 'pouch related behaviour' as well as 'social encounter'. The mean total duration of social behaviour decreased over time for both species and transfer combinations (except for bettong mothers) with various amounts of fluctuation (Fig.6.9).

Values for original bettong mothers increased over the first four weeks following pouch vacation of the young from 3% to 7% of the observation time and subsequently decreased to 5% in the final week. The mean total duration for social behaviour displayed by cross-foster bettong mothers remained constant with results ranging from 1% to 3% of the observation time over the six week analysis period. Exactly the same pattern could be seen in the results of cross-fostered potoroo young, which were only slightly lower compared to original potoroo young and potoroo mothers.

Mean total durations for cross-foster bettong young followed the same trend described by cross-foster potoroo mothers with slightly higher values. Equally high mean total durations for social behaviour were produced by original bettong young with a maximum of 10% of observation time in week 3 and a subsequent decrease to 4% in week 6 after pouch vacation. The results of all young produced for the final week were comparable with the corresponding adult data.

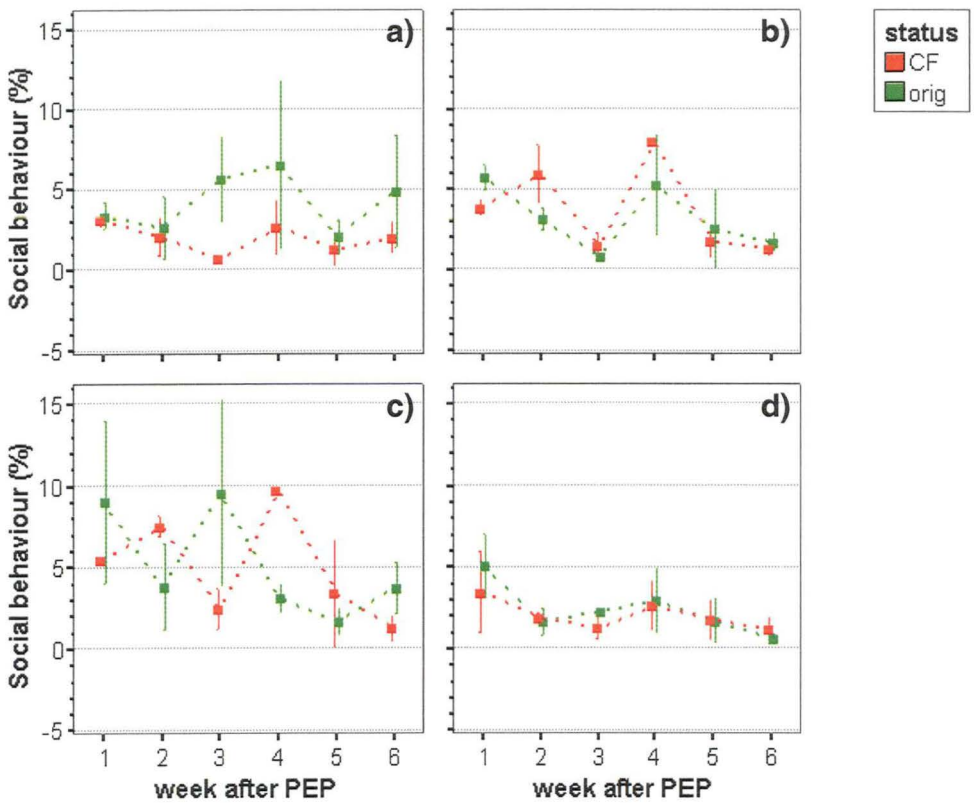

Fig.6.9: Changes in total duration (% of observation) of social behaviour exhibited by bettong mothers (a, N=3/CF, N=3/orig), bettong young (c, N=2/CF, N=3/orig), potoroo mothers (b, N=2/CF, N=3/orig) and potoroo young (d, N=3/CF, N=3/orig) for the six week period following permanent emergence from the pouch (PEP). The status of the actor is displayed in red for cross-foster and green for original animals. Error bars show Mean ± 1.0 SE.

The ratio of total duration and frequency was used to evaluate the quality of social behaviour, since the mean duration of a social event between mother and young could increase even though the total duration of time spent on social activity was decreasing. The ratio (calculated for initial and final week) was declining with time for cross-foster potoroo mothers as well as for all young categories with the exception of original potoroo young initiating social behaviour. Values for the latter category as well as original potoroo mothers and cross-foster bettong mothers remained constant. The ratio increased only for original bettong mothers.

6.3.2.1 Allo-grooming

Allo-grooming was very rarely initiated by young of either species within the observation sessions. The mean total duration of allo-grooming initiated by original bettong mothers increased from 1.6% to 3.6% by week 3 and subsequently decreased to 0.1% by week 5. The decline in the results for cross-foster bettong mothers occurred in week 3 (from initially 1.9% to 0.1%), but stabilized afterwards at ca. 1.3%. Values for original potoroo mothers oscillated around 1.1%, while the results for cross-foster potoroo mothers remained at ca. 0.8% in the second half of the observation period. The initial increase from 1.4% to 6.4% with subsequent decline to 0.1% should not be overrated, since the results were limited to one animal at times and therefore most likely do not represent a general trend. Extensive allo-grooming was initiated by most mothers of both species and transfer combinations after their young were handled by humans.

6.3.2.2 Pouch related behaviour

Interactions with the mothers pouch combined the attempts of young drinking milk or seeking comfort. The mean total duration of pouch related behaviour of original bettong young was high in the initial and third week following pouch vacation (7% of the observation time), but remained low (<=1%) throughout the rest of the analysis period. The amount of data gathered for the other young categories was restricted to certain weeks after PEP (cross-foster bettongs: week 1-3, 4, original potoroos: week 1, 2, 5, cross-foster potoroos: week 1, 2). Results did not exceed 1% of mean total duration in most cases with the exception of 4% pouch related behaviour in cross-foster bettong young in week 5 and 3% for cross-foster potoroo young in week 1, which significantly differed from original potoroo results [$F_{(1, 1)}$=4563.000, p=0.009].

6.3.2.3 Sniffing

Sniffing was a way to establish contact between mother and young. Potoroo mothers as well as potoroo young of both transfer categories showed a decline in mean sniffing frequency over time (Fig.6.10). Initial rates were slightly higher for the mothers (orig: 9 events, CF: 8 events) than for young (CF: 6 events, orig:

4 events), but both initiated sniffing at a rate of ca. 2 events per observation in the final week. Bettong mothers and young showed similar trends with a difference in range of values for the transfer categories. Mean sniffing frequency increased from 9 to a maximum of 13 events per observation for original bettong mothers with a subsequent decrease to 7 events per observation in the final week. A significant difference between the two bettong mother categories was found in week 2 after PEP with original mothers sniffing their young more often than cross-foster mothers [$F_{(1,\ 3)}$=58.389, p=0.005]. Their young showed the same initial increase in sniffing rate (6 to 14 events per observation), but reached peak levels later (week 4) and maintained a relatively high sniffing rate of 9 events per observation in the final week.

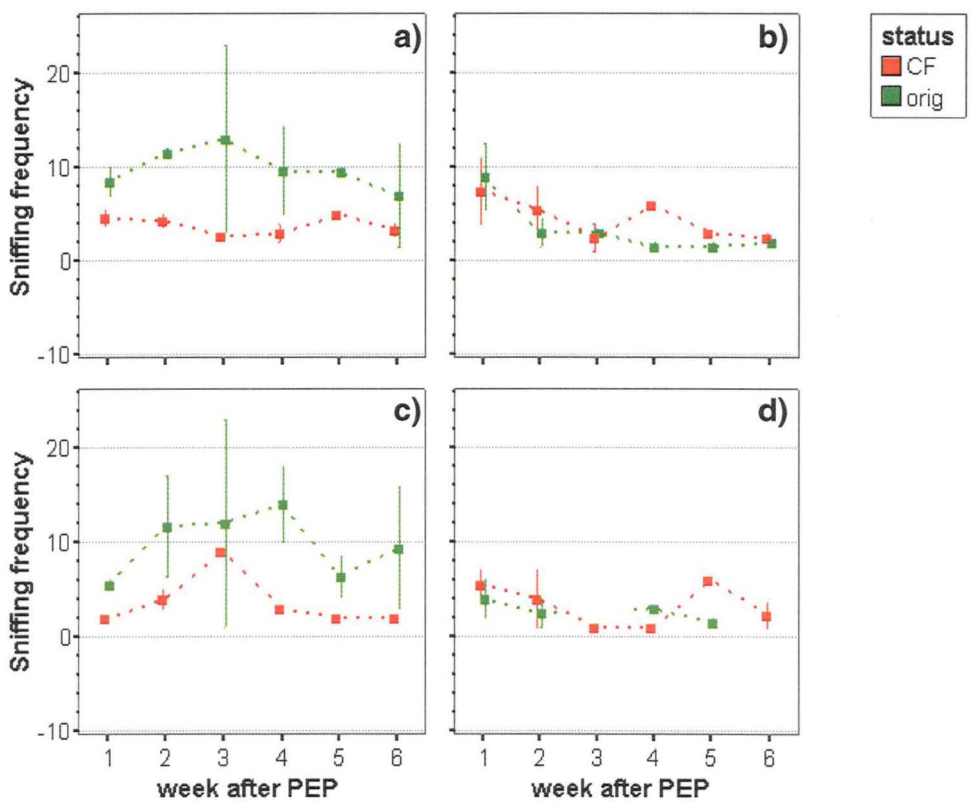

Fig.6.10: Changes in frequency (mean number of events per observation) of sniffing initiated by bettong mothers (a, N=3/CF, N=3/orig), bettong young (c, N=2/CF, N=3/orig), potoroo mothers (b, N=2/CF, N=3/orig) and potoroo young (d, N=3/CF, N=3/orig) for the six week period following permanent emergence from the pouch (PEP). The status of the actor is displayed in red for cross-foster and green for original animals. Error bars show Mean ± 1.0 SE.

The range of values for cross-foster bettong mothers only varied between 3 and 5 events throughout the entire observation period. The mean sniffing rate for cross-foster bettong young remained fairly constant for most of the observation period (between 2 and 4 events) with an isolated peak in week 3 (9 events per observation). Similarities in the change of mean sniffing frequency over time were apparent for all dyad combinations.

6.3.2.4 Following

The behavioural element 'following' was rarely displayed by the mothers. Mean frequencies for original bettong mothers ranged from 3 to 1 event per observation over the observation period and from 4 to 2 events for cross-foster bettong mothers respectively. The following rate for original potoroo mothers remained at ca. 1 event per observation throughout the analysis period (no data in week 3), while a total of only one following event was recorded in week 6 for cross-foster potoroo mothers.

The mean frequencies for young were much higher, in particular for the original bettong young with an initial mean value of 50 following events per observation, which dropped to 16 events in the final observation week. Results for cross-foster bettong young also showed initially higher values (a rise from 7 to 14 events per observation in the first two weeks) and remained constant at a rate of ca. 5 events per observation from week 3 to 6.

Original potoroo young already reached adult levels in week 2, when the initial mean frequency of 13 following events fell rapidly to about 2 events for the remaining observation period (no data for week 4 and 6). After an initial increase in the mean following frequency for cross-foster potoroo young (11 to 20 events per observation), mean values steadily decreased to 6 events in the final week.

6.3.2.5 Approaching

Results for the number of times a mother approached her young covered approximately the same range for both species and statuses with values between ca. 10 and 30 events of approaching per observation session. The mean ap-

proaching rate for original bettong mothers increased with time (from 20 to 31 events per observation), while all other mother categories displayed a decreasing trend (CF bettong: 19 to 10 events, orig. potoroo: 28 to 18 events, CF potoroo: 24 to 13 events per observation). This resulted in a significant difference between the two bettong mother categories in week 6 [$F_{(1, 4)}$=88.200, p=0.001].

The levels for young approaching their mothers were fairly different depending on their status. Values for cross-foster bettong young (varied between 22 and 34 approaching events per observation, maximum in week 2) and original potoroo young (decreased from 35 to 13 events per observation, minimum of 9 events in week 4) corresponded with adult results. The levels for original bettong young and cross-foster potoroo young were much higher when compared to the opposite young status or the adult equivalent. Results for original bettongs ranged from 32 to 63 approaching events per observation with values being two to four times higher than adult results depending on the observed week.

The mean approaching frequency varied even more for cross-foster potoroo young with values ranging from 40 to 75 events per observation, being two to seven times higher than adult results depending on the analysed observation week. Significant differences in mean approaching frequency were found for the two potoroo young categories in week 2 [$F_{(1, 4)}$=11.528, p=0.027] and for the bettong young categories in week 6 [$F_{(1, 3)}$=25.840, p=0.015].

6.3.2.6 Distancing

The results for mean distancing frequencies showed a decreasing trend with time for most mother categories with various degrees of fluctuation. Values for original bettong mothers decreased over the first five weeks from 78 to 55 distancing events per observation, but rose back to the initial level in the final week. Mean values for original bettong mothers were significantly higher in week 4 [$F_{(1, 4)}$=11.236, p=0.029] and 6 [$F_{(1, 4)}$=90.256, p=0.001] compared to results of cross-foster bettong mothers. Mean distancing frequencies for the other mother categories continually decreased with time (CF bettong mother: 39 to 18

events, orig. potoroo mothers: 39 to 15 events, CF potoroo mothers: 42 to 21 events per observation). There was a significant difference between the two potoroo mother categories in week 3 [$F_{(1, 1)}$=300.000, p=0.037] and week 5 [$F_{(1, 2)}$=73.529, p=0.013].

The decrease in mean distancing frequency was only apparent for original potoroo young (31 to 13 events per observation over time, minimum of 6 events in week 4). Mean values for cross-foster potoroo young increased over the first five weeks (32 to 50 events per observation) and subsequently fell to 29 events per observation. Original bettong young followed the trend of their mothers with a decrease of mean distancing frequency over the first four weeks (20 to 12 events per observation) and a subsequent increase towards the end of the analysis period (27 events per observation in week 6). Mean values for cross-foster bettongs initially decreased from 9 to 5 distancing events per observation, but remained mostly constant thereafter at ca. 8 events per observation. This resulted in a significant difference between the two bettong young categories for week 6 [$F_{(1, 3)}$=201.840, p=0.001].

6.3.2.7 'Approach-Distance-Coefficient'

The coefficient for change of distance gives an indication of which animal is responsible for maintaining the proximity between mother (negative values) and young (positive values) (Fig.6.11). In most cases young of both species and statuses were responsible for sustaining proximity to their mothers. Mean coefficient values decreased with time, but appeared higher for bettong young compare to potoroo young. While no obvious differences could be detected for both bettong categories, values for original potoroo young decreased more rapidly over time resulting in negative values for week 4 and 6. A significant difference between the two potoroo young combinations was detected for week 2 [$F_{(1, 4)}$= 27.663, p=0.006].

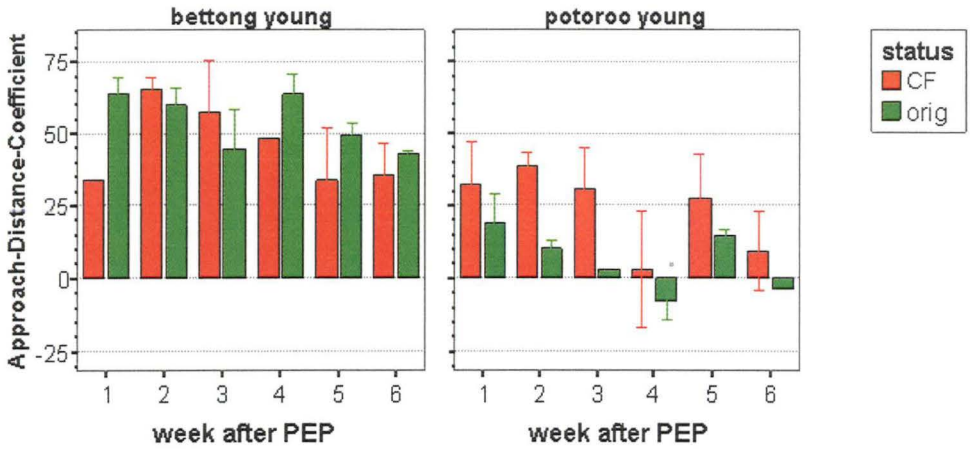

Fig.6.11: Changes in mean coefficient for proximity maintenance between mother and young over time. Values are displayed for young of both species and transfer categories (cross-foster/red, original/green) representing the mother-young dyad (orig. bettong: N=3, CF bettong: N=2, orig. potoroo: N=3, CF potoroo: N=3). Bars show means, error bars show ± 1.0 SE.

6.3.2.8 Social behaviour including others

Although the focus of this chapter lies on mother-young-interactions, the display of social behaviour towards other individuals (Fig.6.12) needs to be mentioned due to the different housing arrangements of the study animals. Bettong mothers and their young (either original [bettong] or cross-foster [potoroo]) were housed separately, while most potoroo mothers and their young were part of a group. The contact opportunities for bettong mothers and their young were therefore restricted to neighbour animals and the presence of vermin in the cage. Potoroo mothers had the additional company of other group members including an adult male and other young at times.

Mean interaction frequencies with other individuals were slightly lower for bettong mothers, original bettong young as well as cross-foster potoroo young compared to other potoroos due to the restricted opportunities mentioned above. Therefore significant differences between the two potoroo young categories found in week 2 [$F_{(1, 3)}$=29.160, p=0.012], week 3 [$F_{(1, 2)}$=270.750, p=0.004] and week 6 [$F_{(1, 2)}$=81.000, p=0.012] should not be overrated. There is an increase in interaction with others for original bettong mothers in week 2 after

pouch vacation of the young, which mainly represented contact with neighbour animals of mostly aggressive nature.

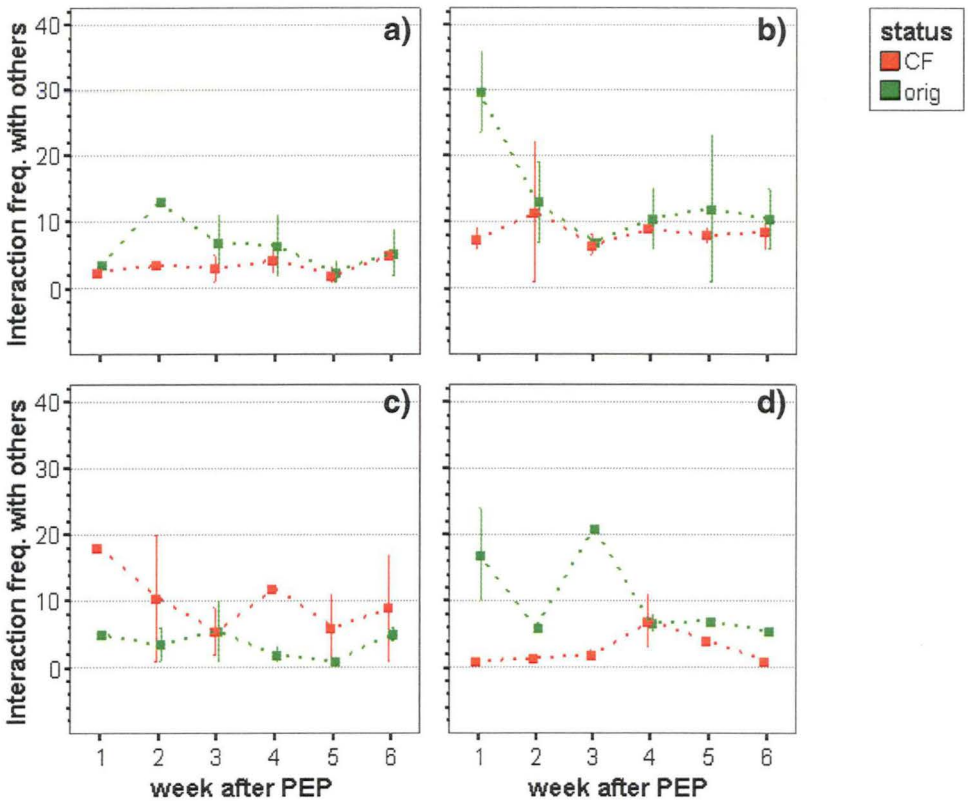

Fig.6.12: Changes in mean interaction frequency with others (number of events per observation) over time exhibited by bettong mothers (a, N=3/CF, N=3/orig), bettong young (c, N=2/CF, N=3/orig), potoroo mothers (b, N=2/CF, N=3/orig) and potoroo young (d, N=3/CF, N=3/orig) for the six week period following permanent emergence from the pouch (PEP). The status of the actor is displayed in red for cross-foster and green for original animals. Error bars show Mean ± 1.0 SE.

Mean results for cross-foster potoroo mothers remained at ca. 9 interaction events with others per observation throughout the analysis period. Initial values for original potoroo mothers (30 events), original potoroo young (17 events, maximum of 21 events in week 3) and cross-foster bettong young (18 events) were much higher, but decreased with time to a comparable level.

Social interactions with other group members are not presented on a detailed level as part of this study. It should be noted though, that cross-foster bettongs

approached other group members (25 events per observation) equally often as they approached their cross-foster mothers (26 events per observation) in the first week after permanent emergence from the pouch, while other young showed a clear preference for their mothers. The results for cross-foster bettongs following their mother or other group members also fell into a comparable range. This trend was also seen in original potoroo young (initial mean frequency was lower: 3 events per observation, no data in week 3 and 6). The data for following other group members in other young categories was limited or non existent for certain weeks after pouch vacation.

Vocal communication within and across the species level was observed mainly between mother and young, but also between the young and other group members (including the male) or animals in neighbouring cages. The calls of the young usually triggered 'approaching', investigation behaviour and/or vocal communication in responding individuals.

Aggressive behaviour towards the associated young was only observed on very rare occasions, but was in most cases accidental since the aggression was directed at a different animal in a neighbouring cage. However, a potoroo transfer mother attacked a cross-fostered bettong young after it climbed through the wire into her cage. She was rearing a cross-fostered bettong young of similar size at the time. Most potoroo mothers exhibited aggressive behaviour towards a male hand-reared potoroo young during the attempt to integrate it into the captive colony. The only potoroo group, which accepted the hand-reared young, consisted of a female, her son and an adult male. The male biased sex ratio in the group did not cause problems, since only the adult male displayed behaviour patterns associated with mating towards the female even when both son and hand-reared male had reached sexual maturity.

No aggressive behaviour was observed between a potoroo and bettongs housed together for several weeks. The potoroo approached and sniffed the bettongs frequently, but his attempts to establish body contact were rejected. Since he was recently separated from his mother, his behaviour could either in-

dicate a generally higher level of sociability (commonly seen in the potoroos of this study) or comfort seeking. The bettongs tolerated the potoroo, but did not initiate social interactions. No mating attempts were observed.

6.3.3 Distance between mother and young

Changes in distance between mother and young were used as a guideline for the growing independence of the young. The individual distance categories are displayed in Figure 6.13 for close proximity (body contact up to 50cm) and Figure 6.14 for further categories (1 to 3m). The mean total duration of time spent in close proximity decreased with time for the mother-young dyads of both species. Mean values for time spent in body contact for bettong mothers with their original or cross-foster young fell from initially 34% to 15% per observation in the final week (cross-foster young) and 12% (original young) respectively. Results for potoroo mothers and their young were lower and showed greater fluctuation. Mean values for mothers with original young decreased from 16% to 1%, while potoroo mothers with cross-foster young showed an initial increase from 14% to 27% (one dyad reached a maximum of 66%) and subsequently decreased to 6% in the final week.

In the 10cm category values for original bettong mothers and their young were higher compared with the other dyad combinations. A mean total duration of 29% of the observation time was spent in 10cm proximity by original bettong dyads in the first week after pouch vacation of young. Values slightly decreased but remained high throughout the first four weeks (maximum mean total duration: 31%) and subsequently dropped to 18% in week 6. Cross-foster bettong mothers and their cross-foster potoroo young spent between 10% and 14% of the observation time 10cm apart for the first five weeks after pouch vacation with a decrease to 5% in the final observation week. This resulted in a significant difference between the two bettong mother categories and their young in week 6 [$F_{(1, 4)}$=19.905, p=0.011]. While values dropped rapidly for original potoroo mothers and their young between week 2 and 3 (11% to 1%), results for cross-foster potoroo mothers and their cross-fostered bettong young decreased steadily over time (13% to 5% of observation time).

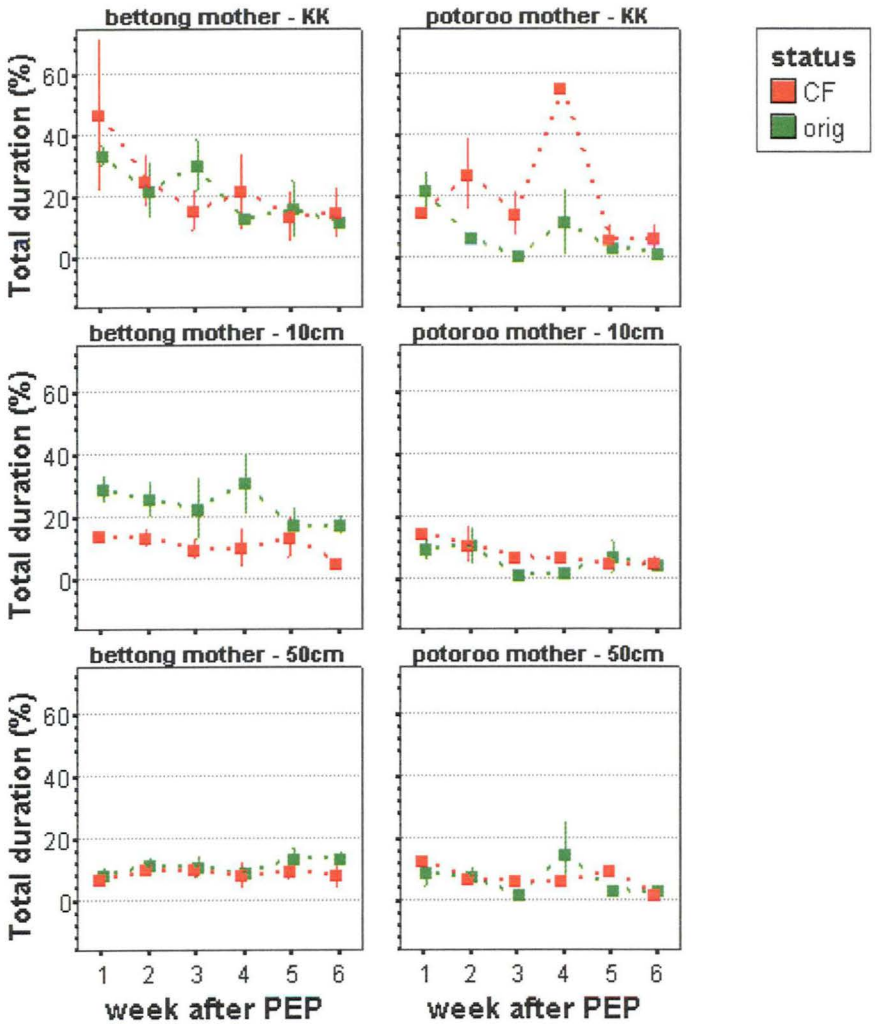

Fig.6.13: Changes in mean total duration (% of observation) of maintained distance between mothers and their young for the categories body contact (first row), 10cm (second row) and 50cm (third row). Values are displayed for mothers of both species (bettong: left column, potoroo: right column) and transfer categories (cross-foster: red, original: green) representing the mother-young dyad (orig. bettong: N=3, CF bettong: N=3, orig. potoroo: N=3, CF potoroo: N=2). Bars show means, error bars show ± 1.0 SE.

The mean total duration results for the 50cm distance category decreased over time for potoroo mothers with young of both transfer types (orig: 8% to 2%, CF: 10% to 2%). Results for cross-foster potoroo mothers and their young were significantly higher compared to original potoroo mothers and their young in week 5 [$F_{(1, 2)}$=70.547, p=0.014]. Values remained mostly constant for cross-foster bettong mothers and their young (ca. 9% of observation time) throughout the entire analysis period. An increase in time spent 50cm apart was apparent

in the results for original bettong mothers and their young over time with values rising from 9% to 14% of observation time.

The mean total duration of time spent further than 50cm apart increased over time for most mother-young dyads at varying degrees. Results in the 1m category for cross-foster potoroo mothers and their cross-foster bettong young remained at ca. 8% throughout most of the observation period with an isolated peak of 12% in week 3. Original potoroo mothers mostly followed the same trend with slightly higher results, while values for both original and cross-foster bettong mothers increased with time (orig: 10% to 14%, CF: 7% to 12% of observation time).

In the 2m category a clear increasing trend is apparent for all mother-young dyads. The mean total duration of time spent at this distance rose from 6% to 20% for original bettong mothers and their young and 12% to 25% of observation time for cross-foster bettong mothers and their young respectively. A significant difference between the two mother categories was detected for week 2 [$F_{(1, 4)}$=13.433, p=0.021]. Results for original potoroo mothers with young were slightly higher (increased from 18% to 32% of observation time), while values for cross-foster potoroo mothers and their young remained at ca. 13% during the analysis period with the exception of an isolated peak level of 32% in week 5. A significant difference between the two potoroo mother categories was found in week 3 [$F_{(1, 1)}$=43440.333, p=0.003].

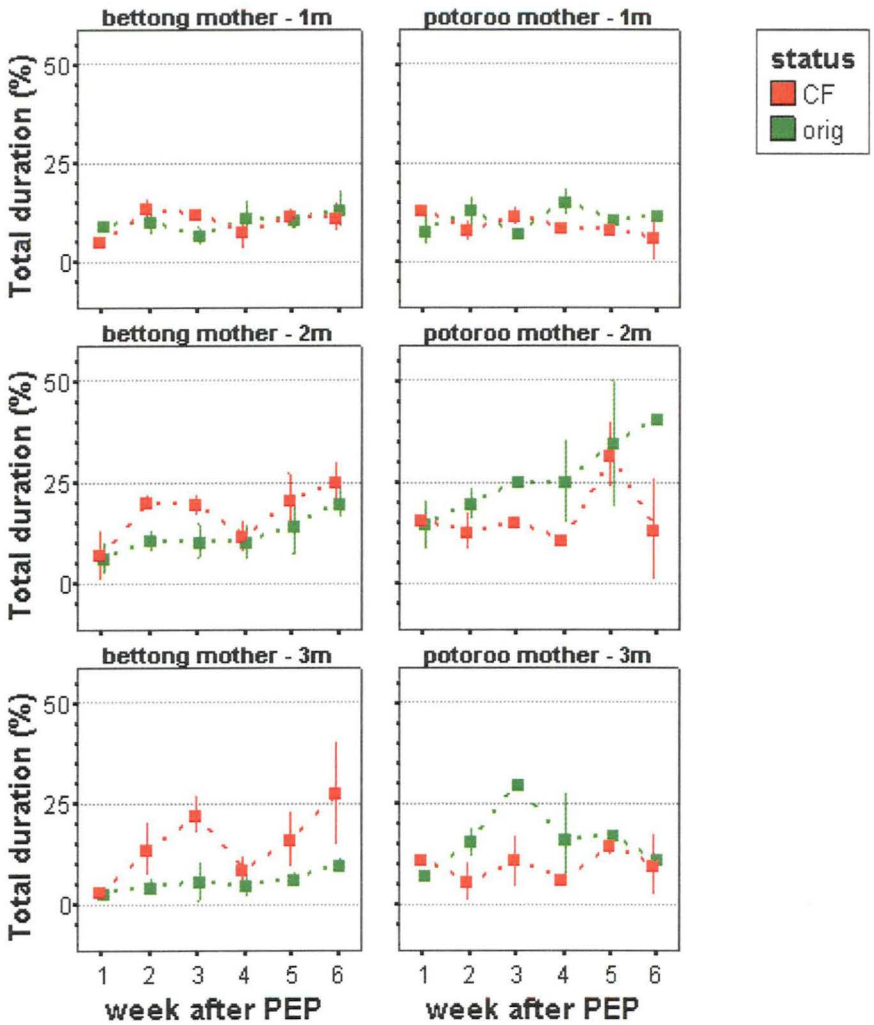

Fig.6.14: Changes in mean total duration (% of observation) of maintained distance between mothers and their young for the categories 1m (first row), 2m (second row) and 3m (third row). Values are displayed for mothers of both species (bettong/left column, potoroo/right column) and transfer categories (cross-foster/red, original/green) representing the mother-young dyad (orig. bettong: N=3, CF bettong: N=3, orig. potoroo: N=3, CF potoroo: N=2). Bars show means, error bars show ± 1.0 SE.

Mean total duration of time spent 3m apart increased rapidly over the first three weeks after pouch vacation of young for cross-foster bettong mothers (6% to 22%) and original potoroo mothers with their young (7% to 30%). Values dropped back in week 4 only to rise again. Original bettong mothers and cross-foster potoroo mothers spent less time at a 3m distance to their young. Mean results for the latter two rose steadily over time from 3% to 10% for original

bettong mothers and 7% to 10% of observation time for cross-foster potoroo mothers and their young.

6.3.4 Difference summary

Table 6.1 summarises all significant differences, which have been detected between the different dyad combinations for the behavioural elements presented above. It is meant to complement the presented data above by giving more detailed information on mean total duration results and/or frequencies if available.

Table 6.1: Significant differences between original (orig) and cross-foster (CF) animals for both species (bet=bettong, pot=potoroo) and actor categories (mother, young or pair). Values are provided for mean total duration (% of observation) or mean frequency (count) for various behavioural elements and distance categories with the associated week after PEP (week).

unit	week	species	actor	F-ratio	p-value	LSD results (Mean ± Standard error)
individual behaviour						
%	3	bet	mother	$F_{(1, 4)}$=12.012	p=0.026	CF (99.02±0.33) > orig (85.48±3.89)
vigilance						
count	5	bet	mother	$F_{(1, 4)}$=14.967	p=0.018	CF (145.67±15.84) > orig (80.00±6.11)
investigation						
%	1	bet	young	$F_{(1, 1)}$=269.695	p=0.039	orig (26.28±0.50) > CF (12.06±NA)
%	3	pot	mother	$F_{(1, 1)}$=337.080	p=0.035	CF (16.69±0.14) > orig (12.28±NA)
digging						
%	2	bet	mother	$F_{(1, 1)}$=272.653	p=0.039	CF (4.39±NA) > orig (0.42±0.14)
locomotion 2ped						
%	1	pot	young	$F_{(1, 1)}$=1466.704	p=0.017	CF (5.72±NA) > orig (0.19±0.08)
count	1	pot	young	$F_{(1, 1)}$=4408.333	p=0.010	CF (62.00±NA) > orig (4.50±0.50)
locomotion 4ped						
%	4	bet	young	$F_{(1, 2)}$=102.223	p=0.010	CF (4.83±NA) > orig (2.85±0.10)
count	4	bet	young	$F_{(1, 2)}$=41.441	p=0.023	CF (57.00±NA) > orig (22.67±2.67)
feeding						
count	4	bet	mother	$F_{(1, 4)}$=10.500	p=0.032	CF (12.00±0.58) > orig (5.00±2.08)
count	5	bet	mother	$F_{(1, 4)}$=10.593	p=0.031	CF (15.67±1.76) > orig (7.33±1.86)
drinking water						
%	3	bet	mother	$F_{(1, 2)}$=77.358	p=0.013	CF (6.22±0.67) > orig (0.28±0.11)
%	6	bet	mother	$F_{(1, 1)}$=363.000	p=0.033	CF (2.94±NA) > orig (0.19±0.08)

(Table 6.1 continued)

unit	week	species	actor	F-ratio	p-value	LSD results (Mean ± Standard error)
auto-grooming						
%	3	pot	young	$F_{(1, 2)}$=89.286	p=0.011	CF (0.20±0.10) > orig (2.06±NA)
%	6	bet	young	$F_{(1, 3)}$=17.999	p=0.024	CF (4.53±0.53) > orig (1.39±0.49)
%	6	pot	mother	$F_{(1, 2)}$=86.843	p=0.011	orig (9.72±1.00) > CF (0.39±0.06)
distance coefficient						
	2	pot	pair	$F_{(1, 4)}$=27.663	p=0.006	CF (38.62±4.66) > orig (10.09±2.78)
10 cm						
%	6	bet	pair	$F_{(1, 4)}$=19.905	p=0.011	orig (17.52±2.40) > CF (5.17±1.38)
50 cm						
%	5	pot	pair	$F_{(1, 2)}$=70.547	p=0.014	CF (9.56±0.56) > orig (3.28±0.50)
1m						
count	3	bet	pair	$F_{(1, 4)}$=21.112	p=0.010	CF (93.33±1.76) > orig (51.33±8.97)
count	6	bet	pair	$F_{(1, 4)}$=16.004	p=0.016	orig (101.67±4.70) > CF (59.67±9.39)
2m						
%	2	bet	pair	$F_{(1, 4)}$=13.433	p=0.021	CF (20.28±1.41) > orig (10.69±2.21)
%	3	pot	pair	$F_{(1, 1)}$=43440.333	p=0.003	orig (25.17±NA) > CF (15.14±0.03)
count	3	bet	pair	$F_{(1, 4)}$=7.763	p=0.050	CF (73.33±10.90) > orig (31.33±10.41)
social behaviour						
count	1	pot	mother	$F_{(1, 3)}$=11.486	p=0.043	orig (172.67±13.37) > CF (113.50±3.50)
count	6	bet	mother	$F_{(1, 4)}$=338.136	p<0.001	orig (136.00±2.52) > CF (46.33±4.18)
allo-grooming						
count	2	bet	mother	$F_{(1, 3)}$=15.000	p=0.030	orig (4.00±0.00) > CF (2.33±0.33)
pouch related behaviour						
%	1	pot	young	$F_{(1, 1)}$=4563.000	p=0.009	CF (3.33±NA) > orig (0.08±0.03)
sniffing						
count	2	bet	mother	$F_{(1, 3)}$=58.389	p=0.005	orig (11.50±0.50) > CF (4.33±0.67)
approaching						
count	2	pot	young	$F_{(1, 4)}$=11.528	p=0.027	CF (75.33±6.69) > orig (29.33±11.78)
count	6	bet	mother	$F_{(1, 4)}$=88.200	p=0.001	orig (31.00±1.00) > CF (10.00±2.00)
count	6	bet	young	$F_{(1, 3)}$=25.840	p=0.015	orig (54.67±1.86) > CF (22.00±8.00)
distancing						
count	3	pot	mother	$F_{(1, 1)}$=300.000	p=0.037	CF (48.00±1.00) > orig (18.00±NA)
count	4	bet	mother	$F_{(1, 4)}$=11.236	p=0.029	orig (57.67±0.88) > CF (24.33±9.91)
count	5	pot	mother	$F_{(1, 2)}$=73.529	p=0.013	CF (36.50±1.50) > orig (11.50±2.50)
count	6	bet	mother	$F_{(1, 4)}$=90.256	p=0.001	orig (77.67±5.70) > CF (18.00±2.65)
count	6	bet	young	$F_{(1, 3)}$=201.840	p=0.001	orig (27.33±0.88) > CF (8.00±1.00)
interaction with others						
count	2	pot	young	$F_{(1, 3)}$=29.160	p=0.012	orig (6.00±0.58) > CF (1.50±0.50)
count	3	pot	young	$F_{(1, 2)}$=270.750	p=0.004	orig (21.00±NA) > CF (2.00±0.58)

(Table 6.1 continued)

unit	week	species	actor	F-ratio	p-value	LSD results (Mean ± Standard error)
interaction with others						
%	3	pot	young	$F_{(1, 2)}$=36.750	p=0.026	orig (2.28±NA) > CF (0.33±0.16)
count	6	pot	young	$F_{(1, 2)}$=81.000	p=0.012	orig (5.50±0.50) > CF (1.00±0.00)
distance others						
count	3	pot	young	$F_{(1, 1)}$=533.333	p=0.028	orig (42.00±NA) > CF (2.00±1.00)
count	4	pot	young	$F_{(1, 3)}$=10.371	p=0.049	orig (11.67±2.03) > CF (2.50±1.50)

6.4 Discussion

Alterations in the behaviour of both mother and young were observed for the transfer dyads; this was also described by Merchant and Sharman (1966). On many occasions the young would either be left behind in the nest or seek shelter during the observation. Johnson (1985) reviewed hiding and following in ungulates and macropods and found it likely that species of similar size or smaller than the red-necked wallaby would show hiding behaviour similar to ungulates, while young of larger species would continuously follow their mothers after final pouch vacation.

The behavioural information previously available for transfer mother-young pairs is very limited and mostly restricted to vocal communication and 'following' behaviour (Merchant & Sharman 1966, Johnson 1981). Altered vocal communication across species has been observed in the present study between young and mother as well as other group members when present. The results for the broader categories, e.g. social behaviour or distance between mother and young, correspond with the outcomes of investigated mother-young interaction in the Tasmanian bettong by Virtue (1987). All study animals exhibited individual behaviour for most of the observation time. Mothers of both species and transfer categories (cross-foster and original) displayed more vigilance behaviour after the young left the pouch permanently, when young were most vulnerable. They also exhibited more investigation behaviour in the early observation period following pouch vacation, which might have served a stimulating purpose for the young to encourage imitation.

The frequency results of biped and quadruped locomotion showed the young's ability of adjusting to non species-specific ways of exhibiting certain behavioural elements, if reared by a transfer mother. Cross-foster young initially imitated the preferred locomotion type of the transfer mother. Gradually they performed their species-specific locomotion type more frequently towards the end of the observation period.

The feeding behaviour in young of both species and transfer categories increased with time due to a decrease of milk supplied by the mother. Drinking behaviour (water) in relation to pouch related behaviour (possibly drinking milk) showed no obvious trends. The performance of auto-grooming remained mostly unchanged for all study animals, however, the rate of over-balancing while auto-grooming in young was much higher for bettong young in the early observation period, especially cross-fostered bettongs. This lack in balance control might be due to the delay in growth and development.

The observed nest building behaviour in subadult cross-fostered potoroo young gave reason to speculate whether the particular behaviour pattern was learnt from their bettong transfer mothers. Although capable, adult potoroos were only observed on rare occasions gathering nesting material in a coiled tail and transporting it to the chosen nesting site. Wallis *et al.* (1997) stated that potoroos do not build nests at all, but rest in an "elaborate depression at the base of trees or shrubs". Although occasionally potoroo study animals have been seen resting in a 'depression' at the base of the door frame or shelter pole, most of the time nest building was observed, even though the structure of the nests was lacking complexity compared to the nests of the bettongs.

Bettong young have been observed gathering nesting material in their first week out of the pouch, which would be indicative for an instinctive rather than a learnt behaviour pattern. It can only be assumed that the nest building behaviour displayed by the cross-foster bettong mother stimulated the performance in the cross-fostered potoroo young. Subadult cross-fostered young were observed frequently gathering nesting material and constructing more complex nests

even after the stimulus (bettong mother) had been removed. It would have been worthwhile investigating if the behaviour was passed down the following generations, but this investigation had to be abandoned due to logistic and time constraints.

The mean total duration of social behaviour exhibited by all study animals decreased with time. Most mothers initially exhibited high distancing frequencies possibly to encourage 'following' behaviour in the associated young. Following is an essential behaviour pattern for a YAF in the wild, since it is not allowed to seek refuge in the pouch anymore and therefore has to rely on its mother to lead it away from danger when alarmed (Dawson 1995).

The young were responsible for maintaining proximity to their mothers throughout most of the observation period. The active role in the spatial organization of mother and young is well documented for a variety of species within the Macropodoidea, e.g. *Macropus eugenii* and *Megaleia rufa* (Russell 1973), *Macropus rufogriseus banksianus* (Johnson 1985), *Macropus robustus* (Croft 1981), *Macropus giganteus* (Stuart-Dick 1987) and *Bettongia gaimardi* (Virtue 1987). However, the results for original potoroo dyads in the present study showed that mothers more actively participated in proximity maintenance towards weaning, possibly indicating a generally higher level of sociability in potoroos. Russell (1989) highlighted that the decrease in social interaction frequencies and increase of distance between mother and young with increasing age of the young should not be mistaken for a lack in the mother's awareness of her young and its position.

All mother-young pairs showed a decreasing trend over time for the combined distance categories for close proximity (body contact, 10cm and 50cm) and an increasing trend for the combined remaining categories (1m, 2m and 3m). However, the obtained results are under-representative for original potoroos, since potoroo young had spent a majority of the observation time in body contact with another group member, a category not included in the described mother-young behaviour. Potoroo young were frequently observed to establish

body contact with either their mother or another group member if present when performing individual behaviour (e.g. auto-grooming, vigilance or investigation). This seeking of a literal 'back up' while performing maintenance behaviours could indicate a lack of independence in the young or support the higher degree of sociability in potoroos in general compared to bettongs.

The decrease in exhibited social behaviour combined with the increase in distance between mother and young with time was used as an indicator for the growing independence of the young. This independence strongly relied on the knowledge of the terrain and possibly the presence of the mother, since young, which were moved with their mothers into larger rehabilitation cages, did not display independent behaviour in the unknown environment, but instead followed their mothers closely (Gates pers.comm., this study).

After their handling by humans, bettong mothers have been observed on rare occasions to attack their YAF (this study). Williams and Williams (1999) have also described this behaviour for the Tammar wallaby with fully furred pouch young and came to the conclusion that this behaviour pattern was odour related. Their suggestion was to return the young to the pouch and subsequently tape the pouch with masking tape to give the mother the opportunity to calm down while the human odour was eliminated from the young. Herd (1988) achieved a 100% success rate in a foster experiment with beef calves by placing hessian sacks on the calves for four days prior to the transfer procedure. Subsequently, the sacks were swapped so the transferred calve carried the scent of the natural calve when placed with its new mother . The 'wrong' odour might have been a possible cause for the high rejection rate of fully furred pouch young in Johnson's (1981) preliminary study on cross-fostering. It might also explain the observed aggressive behaviour towards a cross-foster young from a neighbouring group and a hand-reared young observed in this study. However, it is probably not the only clue required for kin-recognition, since most females showed extensive allo-grooming instead of aggression when being reunited with their young after handling by humans.

Close and Lowry (1990) did research on marsupial hybrids and stated that learnt behavioural species-specific differences between potential hybridising species could be avoided by cross-fostering the pouch young into the pouch of the species they would form hybrids with later. Learning non-species-specific behaviour would be a disadvantage for cross-foster young in breeding programs for conservation, since they could not be released back into the wild once mature. This, however, requires precise knowledge of the entire behavioural repertoire of each species to distinguish between learnt non-species-specific behaviour and stimulated behaviour pattern that are usually performed on a rare basis (e.g. above described nest building behaviour in potoroos). Although the transfer animals of this study initially appeared to adjust their behaviour to that displayed by their cross-foster mothers, they developed species-specific behaviour patterns regardless long-term.

However, problems were experienced with reuniting the cross-foster young with their own species after weaning (3.3.5 Reunion with original species), which indicates some degree of learning on a social level given the different social systems the young grew up in (mother-young isolated [bettongs] versus mother-young integrated in a social group [potoroos]). This did not lead to unintended cross-breeding, which has been reported for Tammar and Agile wallabies living together in captivity (Williams & Williams 1999). The social integration problems of cross-foster animals in this study were resolved by careful grouping of subadult individuals, but highlighted a potential general problem of the cross-fostering technique when choosing a transfer species with a different social system.

Chapter 7: Final discussion

The potential of assisted reproduction technologies for conservation management of mammalian species in captivity has been reviewed by several authors (Rodger [1990], Tribe *et al.* [1994], Taggart *et al.* [1997], Bainbridge & Jabbour [1998], Mate *et al.* [1998], Johnston *et al.* [1999]). The reviewed techniques are well established in husbandry of domestic animals and treatment of human infertility (Rodger 1990); however, the aims for endangered species are quiet different from those for domestic species in terms of preventing artificial selection and genetic adaptation to captivity (Bainbridge & Jabbour 1998).

Marsupials and monotremes have no domestic equivalent and although the Tammar wallaby (*Macropus eugenii*) and the Brushtail possum (*Trichosurus vulpecular*) appear to be appropriate models for most macropod and possum species, species-specific reproductive differences need to be considered for a successful application of artificial breeding technologies (Johnston *et al.* 1999). A pro-active approach needs to be taken for trialing procedures before remaining population numbers are too low (Tribe *et al.* 1994, Johnston *et al.* 1999).

Since every technique cannot be discussed in detail within the context of this thesis, only important aspects of the commonly used technologies are presented to provide information for the judgement of cross-fostering against the background of available techniques. Artificial insemination (AI), which comprises collection, preservation and insemination of semen, has been performed with considerable success in conservation programs, however the control over the female's genetic contribution is limited to selecting her for insemination (Bainbridge & Jabbour 1998). While urine contamination (Bainbridge & Jabbour 1998) and possible constipation in the donor male after prolonged electric stimulation have been reported as negative side effects, electroejaculation is commonly used and considered to be a reliable and relatively non-invasive technique of semen collection (Tribe *et al.* 1994) next to the use of artificial vaginae and postmortem collection. The latter in particular allows the rescue of genetic material from recently deceased, rare and endangered species (Taggart

et al. 1997) as well as from animals that needed to be euthanased unexpectedly (Bainbridge & Jabbour 1998). Harvested genetic material, which can also include fertilised eggs or embryos, can be potentially stored indefinitely in liquid nitrogen and therefore allow national and international transport to widely separated breeding groups as well as the establishment of a long-term genetic bank (Rodger 1990). Johnston *et al.* (1999) pointed out the benefit of transporting preserved genetic material opposed to live animals in terms of animal welfare concerns, cost savings, increased amount of sent genetic material at a time and less quarantine difficulties.

The possible investigation of genetic material has less ethical implications for the embryos of endangered species opposed to humans (e.g. 'desirable' genotypes, diagnosis of genetic disease), since it has to be assumed that the captive species carries all the genetic information required for survival in the wild, which should therefore be best left unchanged (Bainbridge & Jabbour 1998). However, the latter authors highlighted the benefit of selecting genetic material on the basis of chromosomal sex for achieving an optimal sex ratio in small populations. Johnston *et al.* (1999) pointed out the prospect of screening semen for infectious agents prior to use in an artificial insemination program. The authors referred to unpublished observations by Timms and Johnston describing the detection of *Clamydia sp* in Koala seminal plasma. The careful management of genetic material is vital for successful breeding programs and should consist of a captive population and a gene bank in constant dynamic interaction (Bainbridge & Jabbour 1998).

Rodger (1990) highlighted that the control of the female's reproduction has to be the primary aim of artificial reproduction technologies, since the female's physiology and behaviour are the limiting factors of production. Mate *et al.* (1998) pointed out that natural methods such as pouch young removal did not guarantee a sufficiently precise timing of subsequent developmental events.

The artificial control of ovulation rate, however, not only allows the collection of a maximised number of embryos per donor female, but also establishes control

over the female's genetic contribution to future generations (Bainbridge & Jabbour 1998). The timing of mating, ovulation and fertilisation can be regulated via superovulation, but protocols developed for marsupials still need to be customised to the species of interest (Mate *et al.* 1998).

Once multiple embryos are produced via natural mating or artificial means, they can then be implanted into a surrogate mother for subsequent development to term (Rodger 1990). Bainbridge and Jabbour (1998) referred to females of related common species as 'incubators' for endangered species in inter-species transfers, that are particularly useful when the number of breeding females in small relict populations is the limiting factor for reproductive success. The latter authors, however, also reported low pregnancy rates following inter-species embryo transfer in eutherians, which might be due to a physiological mismatch of both species. They suggested artificial chimaeric embryos as a potential solution.

Marsupials offer the advantage of a short pregnancy with subsequent non-surgical access to the young resuming development in the pouch. The rare but possible production and rearing of twins in monotocous species suggests that each uterus can accommodate at least two embryos (Mate *et al.* 1998). The number of surviving young is, however, limited by the number of teats in the pouch, which the young have to attach to (Rodger 1990, Mate *et al.* 1998). The latter authors also referred to possible pouch crowding problems in the more advanced stage of young development, which could be avoided by transferring pouch young to other recipient females.

Pouch young transfers have been mainly performed with members of the superfamily Macropodoidea. Merchant and Sharman (1966) performed intra-species transfers (Red kangaroo, Grey kangaroo) and inter-species transfers (Red kangaroo x Grey kangaroo, Tammar wallaby x Red kangaroo, Swamp wallaby x Red kangaroo, Red kangaroo x Red-necked wallaby, Yellow-footed rock wallaby x Red kangaroo). Clark (1968) fostered Red kangaroos followed by Johnson (1981), who cross-fostered several wallaby species (Whiptail or

Prettyface wallaby x Agile wallaby, Spectacled hare-wallaby x Plain rock wallaby, Bridled nail-tail wallaby x Plain rock wallaby, Swamp or Black-tailed wallaby x Agile wallaby). Bell and Close (1994 as quoted in Taggart *et al.* 1997) transferred rock wallaby pouch young to Tammar wallabies. Trott *et al.* (2003) have performed recent intra-species transfers in the Tammar wallaby. Jones *et al.* (2004) cross-fostered Black-footed rock wallaby pouch young (*Petrogale lateralis* 'MacDonnell Ranges race') to Tammar wallaby mothers.

Intra-species transfers of rat-kangaroos have been conducted by Rose (1986; Tasmanian bettongs) and Smith (1989; Brush-tailed bettongs). Smolenski (1986) performed inter-species transfers between Tasmanian bettongs and Long-nosed potoroos. A reference for pouch young transfers in koalas was mentioned by Tribe *et al.* (1994) as a personal communication by Douglas.

Conservation orientated cross-fostering has been performed by Smith (1998, Northern bettong x Brush-tailed bettongs) and Taggart *et al.* (1997, Black-footed rock wallaby x Tammar wallaby; Brush-tailed rock wallaby x Tammar wallaby quoted as unpublished data of Taggart, Underwood and Holtz). Horsup (1999) described intra-species transfers within the Southern hairy-nosed wombat, which are currently trialed with the aim of cross-fostering the endangered Northern hairy-nosed wombat to Southern hairy-nosed wombats and possibly human carers. Temple-Smith (2003) quoted unpublished data of Taggart and Temple-Smith of successful pouch young transfers of wombat pouch young between Southern hairy-nosed wombats.

Cross-fostering has a great potential for the conservation of marsupial fauna, since it probably can be used for any species as long as a suitable closely related common species is available. The advantage of pouch young transfers over most other artificial breeding techniques is its non-invasive and simple nature. The procedure can be performed as early as day one of pouch life with little expenditure for the donor female that is freed from the energy drain of lactation and able to produce more offspring. The transferred pouch young is easily accessible in the pouch throughout most of its development.

Cross-foster techniques also have the potential of extending laboratory technologies to the field via pouch young isolation, which enables the establishment of captive breeding colonies without removing valuable breeding adults from the wild (Taggart *et al.* 1997, Taggart *et al.* 2002). Early experiments allowed the transport of pouch young over great distances before transferring them to the recipient female (Merchant & Sharman 1966, travelling distance 110 miles; Johnson 1981, travelling time 4-30 hours). Taggart *et al.* (2002) pointed out the importance of combining pouch young isolation with cross-fostering techniques for conservation purposes by only bringing the pouch young of threatened macropod species into captivity and with their removal simultaneously activating the diapause embryo in the wild-based donor mothers, hence accelerating reproduction in the wild. This would also by-pass the problem of poor breeding records of many marsupial species once in captivity, which are probably linked with high stress levels and/or inattention to natural species-specific breeding strategies (Taggart *et al.* 1997). Bradley *et al.* (1999) have defined the knowledge of biology and reproduction of species, husbandry expertise and consideration of the genetic implications as key requirements for the establishment of captive breeding programs. Although these requirements appear to be straightforward, the lack of basic reproductive knowledge for most marsupial species is probably mainly responsible for the slow progress of artificial reproduction next to the lacking financial support (Tribe *et al.* 1994).

The application of artificial reproduction technologies to domestic species is highly subsidised due to demand driven by human consumption while the low funded application to endangered species purely attempts to ensure their continued existence. The unbalanced distribution of financial support stresses the lack of acknowledgement of the severity of extinction in the 'bigger picture' of biodiversity.

Although the idea of using cross-fostering in marsupials for conservation purposes dates back several decades (Merchant & Sharman 1966), most of the following literature (Clark 1968, Johnson 1981, Rose 1986, Taggart *et al.* 1997, Smith 1998) only focused on the possible acceleration of the female reproduc-

tive rate and the acceptance of transferred young by mothers of different species. There is little published on the survival of the transferred young up to independence and its future reproductive success. Without ensuring the young's long-term well-being and capability of exhibiting species-specific behaviour including mate recognition as well as the successful rearing of own offspring, the quality of captive breeding programs and possible reintroductions into the wild are questionable.

As part of this thesis intra-species and inter-species transfers were performed with Tasmanian bettong and Long-nosed potoroo young. Mothers of both species were capable of successfully rearing their transferred young, however survival and growth rate favoured cross-foster potoroo young. The 'time window' for transfer age appeared to be limited by a TAD of three weeks due to physical constraints (teat size in relation to mouth opening) as well as the occurrence of growth abnormalities. The latter were probably due to an inability in utilizing nutrients by younger transferred animals, while older young were deprived of the nutritional level their growth and development normally requires, resulting in retarded growth and possible brain damage.

Johnson (1981) advised against transferring young during late pouch life due to a high rejection rate observed in his preliminary study. No transfers of fully furred young about to leave the pouch were conducted as part of this thesis, since the savings of time and energy for the mother were not considered sufficiently economical, if the young was removed shortly before pouch vacation when milk production peaks. Transfers during early pouch life were considered preferable by giving the young the opportunity to adapt slowly, thereby reducing the chance of rejection.

Mortality in transferred young occurred in early as well as mid pouch life with death taking place either shortly after transfer or up to several months later. The loss of young soon after the transfer procedure was most likely due to a re-attachment failure, while the death of older young was probably related to inappropriate nutrition. The results of the present study supported the recent

findings of Trott *et al.* (2003), since pouch young transfers appeared to have no obvious influence on milk composition and milk production rate. Therefore foster young of asynchronous transfers as well as cross-foster young were provided with inadequate milk composition for their age. Younger foster animals appeared to benefit from the more advanced milk and showed accelerated growth, while older foster young fell behind in their growth and development.

Cross-foster young were provided with milk for a different species in addition to a different age and developmental stage. All cross-foster potoroo young were between one and three weeks older than the bettong young they were transferred with and they still appeared to have an advantage in growth and development compared to untransferred potoroo young. The younger age of the cross-foster bettong young did not result in a growth advantage due to lesser quality and possibly quantity of the provided milk. When cross-foster bettong are compared with untransferred bettong young, the cross-foster young are growing at a "potoroo rate", which means that they will have a longer pouch life and therefore an altered growth rate. However, the surviving cross-foster bettongs were not only slower in their growth and development, they showed severe signs of lacking muscle development. Their appearance was best described as "just skin and bones". In spite of this, they did survive unlike most of the other cross-foster bettong young. A cross-foster bettong transferred early in pouch life with a potoroo young of the same age eventually failed to adjust to the different milk composition and was lost nine weeks after the procedure. Using knowledge of the mother's milk composition and/or the young's growth rate one can strategically employ the TAD for the benefit of the transferred young's survival and development.

Earlier studies (Merchant & Sharman 1966, Johnson 1981), which included behavioural aspects of the foster and cross-foster mother-young dyads, focused mainly on the maternal behaviour rather than both mother and young. In the present study, vocal communication was observed between mother and young of both species; however, the fact that other animals (including males) also re-

sponded to calls of the young might indicate a universal rather than a species-specific quality of the call or parts of it.

The behavioural data gathered for this thesis suggested that some cross-foster young altered their behaviour initially, but developed species-specific behaviour patterns regardless. All surviving matured young were successful in mate recognition, offspring production and nurturing (applies to female young only). Problems were experienced when integrating cross-foster bettong young with their own species after weaning, probably due to different levels of sociability in the two species. This issue was addressed by pairing the cross-foster males with inexperienced females to facilitate an easier transition process from sub-adult to adulthood.

Several problems that occurred during this thesis have led to a set of recommendations, which should be incorporated into current management practices to ensure best possible care and breeding success.

- Good husbandry practices are essential for preventing disease within the captive colony and should be examined closely if breeding problems occur (e.g. due to over-crowded cages [Tyndale-Biscoe 1968, Maynes 1973], failure to rotate potential breeding partners, insufficient artificial diet). Poor housing conditions can also negatively impact on milk production rates (Akers 2002).

- Environmental enrichment is usually seen as a trade off between the animal's needs and the researcher's aims. Although the animal's welfare has to be first priority, it also reflects on the quality of the gathered data at the same time. Environmental enrichment is vital for exhibiting stimulus driven behaviour. Potoroos, for example, tend to create a network of runways in the thick groundcover of their natural habitat (Watts 1993). If this type and amount of vegetation is not provided in captivity such tunneling behaviour will not be observed. Carlstead (1996) defined environmental enrichment as an attempt to provide the animal with a com-

plex environment to increase the possibility that the captive animal's own behaviour will satisfy its needs.

She also pointed out that animals with more behavioural options will be better equipped in dealing with stressors or alleviating boredom. The provision of an adequate environment therefore does not only encourage more 'natural' behaviour and maintenance of physical condition, it also provides the researcher with better quality data. An inadequate environment will encourage the establishment of stereotypic behaviour, abnormal behaviours (e.g. copulation with inappropriate partners or objects) and behavioural deficiencies (Carlstead 1996). The latter in particular would have a negative effect on breeding programs and possible reintroductions to the wild (Thompson 1996), which would make the attempt to cross-foster animals in the first place questionable.

- Animals should be housed in appropriate social groups for their species in order to encourage species-specific behaviour (reintegration problems are addressed above).

- Mothers should not be considered as "breeding machines", but given the chance to rear their own offspring on an alternating basis. Especially, first time mothers should be given the opportunity to explore their maternal potential (e.g. losing the first pouch young or experiencing a mother-young bond from the mother's perspective) before being utilized as a potential transfer mother. Females with good maternal behaviour are more successful in rearing offspring and this should apply to cross-foster young as well (Ashworth 1996 reviews strategies of maternal investment in marsupials).

- Animals should be given the opportunity to exhibit species-specific behaviour, but just as important is the knowledge of such behaviour patterns by the caretakers to enable possible intervention. An indication of an aggressive conflict, for example, could be the sighting of nocturnal

animals feeding during the day (the dominant animal may be restricting access to food). The observation of neglectful maternal behaviour is an important factor in addition to body weight loss in the young for implementing hand-rearing procedures.

- Hand-rearing has been introduced earlier as a possible support procedure for the cross-fostering technique if rearing difficulties are present. Hand-rearing is often associated with the issue of possible imprinting (Primack 1995), which also applies to the cross-fostering technique, hence the test of mate recognition. Hand-reared animals are also often classified as "too tame" and therefore not suitable for release. The tameness, which was also observed in the generations of bettongs and potoroos being born in captivity, can be used as an advantage, since handling and close proximity to humans in general appears to be less stressful, which makes the tame females better potential transfer mothers.

  The concern of not being suitable for release is refutable if there is strategic release planning. While breeding stock remains under controlled conditions in captivity, offspring is released in stages into larger enclosures to encourage self-sustainability and species-specific behaviour before weaning them of human support when finally released back into the wild or protected areas (Jackson, Earth Sanctuaries Limited, pers. comm.). This weaning process might take several generations depending on the species, but will insure self-sufficiency in the animals and break possible bonds between them and their caretakers.

In addition to recommendations, this project raised several new questions. Since the survival and flourishing of transferred young is vital for the successful implementation of cross-fostering techniques for the conservation of endangered species, a worthwhile field of further study would be the direct environment of the transferred young in relation to mortality rates in early pouch life. This would include, for example, possible influences of the young's ability to detect different pouch scent or milk taste on attachment success or possible

age related changes in the transferee's capacity to digest milk. The effect of asynchronous pouch young transfers on immunity transfer via milk could be examined as well as the influence of stress on milk composition and production rate in recipient mothers to determine a possible relationship with the mortality rate in transfer young. Non invasive techniques such as electrical impedance measurements to measure fat reserves could be very useful for further studies of body condition and development of transferred young as well as techniques to measure steroid hormones in faeces and urine to determine endocrine function and puberty in transferees. Behavioural studies should be given more importance for gaining a better understanding of the mother-young interactions throughout the entire lactation process, the determination of instinctive and learnt behaviours in transferees, the implications of different levels of sociabilities in the transfer species, the reproductive capabilities of matured offspring and rehabilitation requirements for a possible release of transferees and/or their offspring into the wild.

Cross-fostering has proven to be a successful technique in the field of assisted reproductive technology, which accelerates the females reproductive rate, but also provides much control over the growth and development of the transferred young by careful planning of the transfer procedure (e.g. combination of appropriate species, temperament and experience of the recipient mother; age of young at time of transfer and TAD of transferred young). The accessibility of the young in the pouch enables progress monitoring and, where required, early intervention in terms of repeated transfer and/or hand-rearing.

However, researchers have to face the ethical confrontation, which goes hand in hand with the application of all *ex situ* strategies, by determining if such actions are in the best long-term interest for the species concerned despite loss of natural habitat (Primack 1995). More importantly, an integrated approach has to be implemented to secure long-term survival of any target species, which combines exclusion zones, captive breeding programs, effective pest control and habitat management (controlled burns, weed and erosion control), disease investigation, reintroduction programs for plants and animals as well as public

education (Blyde 1999). Successful *ex situ* strategies only represent a possible application of a highly specialised technology, but not a conservation success (Western & Pearl 1989) – an interdisciplinary approach is the only realistic chance for long-term survival for any species of this planet.

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Appendix

A.1 Native plants

Acacia riceana	Rice Wattle
Allocasuarina littoralis	Bulloak
Allocasuarina verticillata	Coastal Drooping Sheoak
Eucalyptus barberi	Barber's Gum
Eucalytus delegatensis	Alpine Ash, Gum-Topped Stringybark
Eucalyptus perriniana	Spinning gum
Baloskion tetraphyllum	Tassel Cord Rush
Carex longebrachiata	Drooping Sedge
Cyperus lucidus	Leafy Flat Sedge
Gymnoschoenus sphaerocephalus	Button Grass
Lomandra longifolia	Sagg
Dianella tasmanica	Tasman flax lily
Poa labillardieri, Poa sieberiana	Poa grass

A.2 Anaesthesia settings

No pre anaesthetic was administered, since animals remained reasonably calm during the commencement of the anaesthesia procedure when kept in the dark bag. Isoflurane was initially given at a high dose rate to minimise the duration of exposure to the anaesthetic for the animal. It was subsequently regulated down depending on the needs of the individual. The breathing rate was usually used as an indicator for the level of alertness. Once the rate decreased, the animal was removed from the bag to monitor vital signs and commence the planned procedure (e.g. milking, pouch young transfer). However, some potoroos with high stress levels were observed to hold their breath at the beginning of the anaesthesia procedure, which subsequently led to complete breathing failure while under the influence of Isoflurane. They were given pure oxygen to breathe while their torso was massaged to encourage breathing. None of these animals were lost, but as a result of the above problems the Isoflurane dose rate was gradually increased for potoroos instead of starting with a high dose.

A.3 Toxoplasmosis

The autopsy of a potoroo young-at-foot revealed toxoplasmosis as the cause of death. To detect if toxoplasmosis was spreading through the entire breeding colony, blood samples were taken from both bettongs and potoroos, including the mother of the dead young (female '330C'). Four out of five of her pouch young died at an early age. However, the medium sized pouch young, she carried when brought into captivity, did survive. He was a fully grown adult at the time of blood testing. No bettongs were infected and only some potoroos carried *Toxoplasma gondii*. The carrier animals were wild caught, including female '330C' and her adult son. Since he was only a medium size pouch young when his mother was captured, it appeared very unlikely that he had much contact with the environment outside the pouch at that stage. The fact that he was an adult carrier, having grown up in an area free of toxoplasmosis, suggests that *Toxoplasma gondii* has been transferred from mother to son via the milk. This has been found in other species (Barry Munday, pers.comm.).

A.4 Data collection sheet

Displayed below is an example of a data collection sheet for the milking procedure and growth measurements of associated young. The circles next to the milk sample information were used for indicating which teat the young was taken off.

Date:

♀ cage:				milk sample ○ ○ ○ ○		
pouch temperature: °C						
ID:					PY	YAF
weight:				number		
isoflurane: time:				amount		
oxytocin:						
	PY			YAF		
sex						
foot left						
foot right						
tail						
head						
weight						
age						

notes:

A.5 Calming effects

An artificial heartbeat (ChilsonRoth LLC., Colorado, USA, taken out of a SnuggePuppie$_{tm}$, Snuggle Pets, NSW) was placed into the humidicrib, which seemed to have a soothing effect on the pouch young during the three hour separation period from their mothers for milk sample collection. A recording of bird sounds was played during the time the animals were kept in the laboratory (Listening Earth, Andrew Skeoch & Sarah Koschak, LECD9501/Tall Forest, LECD9601/A morning in the Australian bush) to help create a calm atmosphere among the stressed animals, but as with the artificial heartbeat mentioned above, appropriate test trials are needed before making recommendations.

A.6 Standard curves

Standard curves were produced at the beginning and end of each assay.

Protein assay: Bovine serum albumin (BSA) was used as a standard for the Biuret method. A stock standard (SS) was prepared by dissolving 0.2g BSA in 1mL of distilled water. Diluting the stock standard with distilled water according to the concentration required for the standard curve produced the working standards (WS). In accordance with the described four-step protocol, 25µl of WS were processed instead of the milk sample (4.2.2.1 Protein Analysis). The blank consisted of 25µl of distilled water, 1µl of Lipase, 25µl of Na_2EDTA and 1.5ml of Biuret reagent.

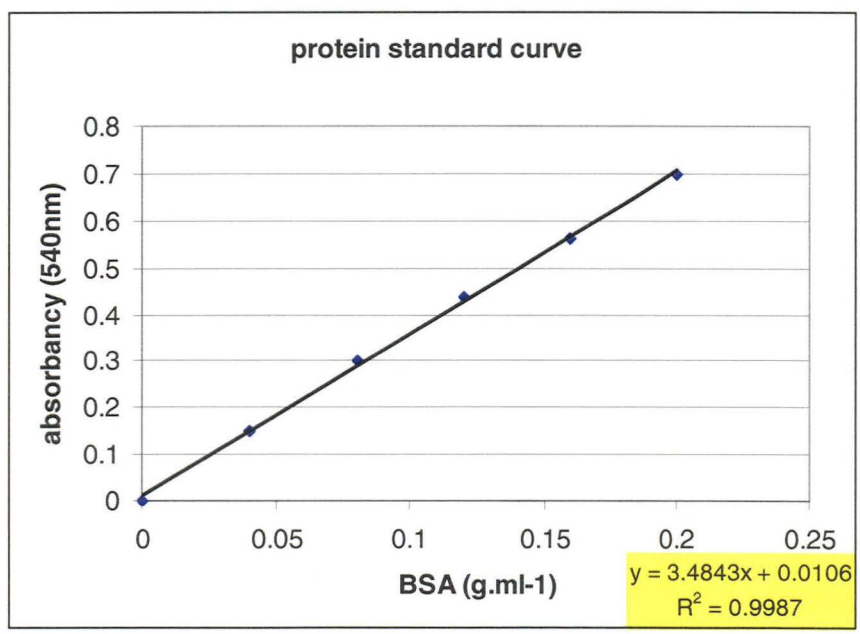

Fig.A.6.1: Protein standard curve, N=6

Carbohydrate assay

Milk Sugar, α-Lactose (SIGMA) was used as a standard for the carbohydrate assay outlined under 4.2.2.2 Carbohydrate Analysis. The milk sugar was dissolved in distilled water while being placed in a water bath set at 50°C (SS: 0.2g α-Lactose/1ml distilled water). In accordance with the described procedure 5µl of WS instead of milk were diluted with 2ml of distilled water. A total of 200µl distilled water combined with 1ml of 3.55% (w/v) phenol solution and 3ml of concentrated sulphuric acid served as the blank.

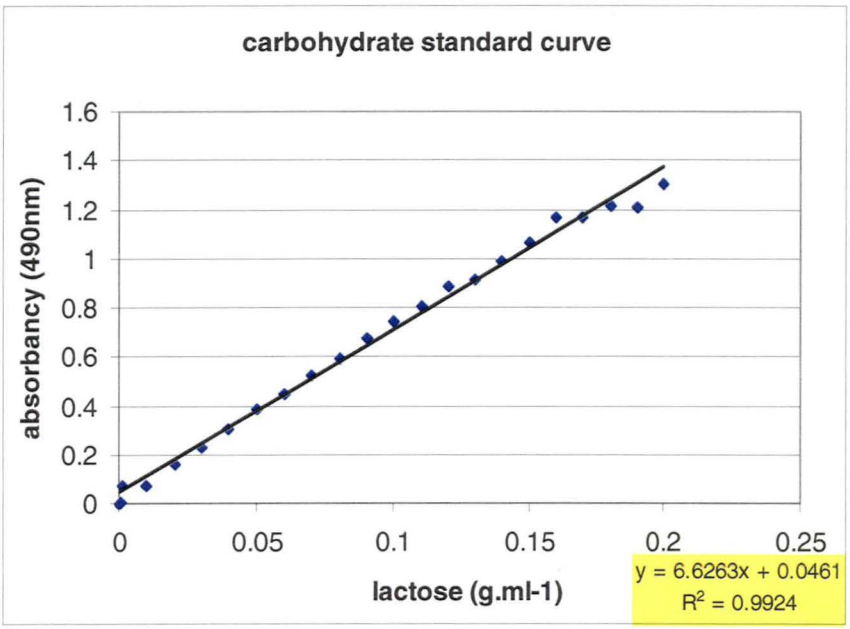

Fig.A.6.2: Carbohydrate standard curve, N=22

Lipid analysis

The lipid content was initially determined using the creamatocrit method and subsequently standardised using the Roese-Gottlieb ether extraction technique in cooperation with the Chemistry department of the University of Tasmania. The linear regression of the methods is displayed below for the samples of both species separately.

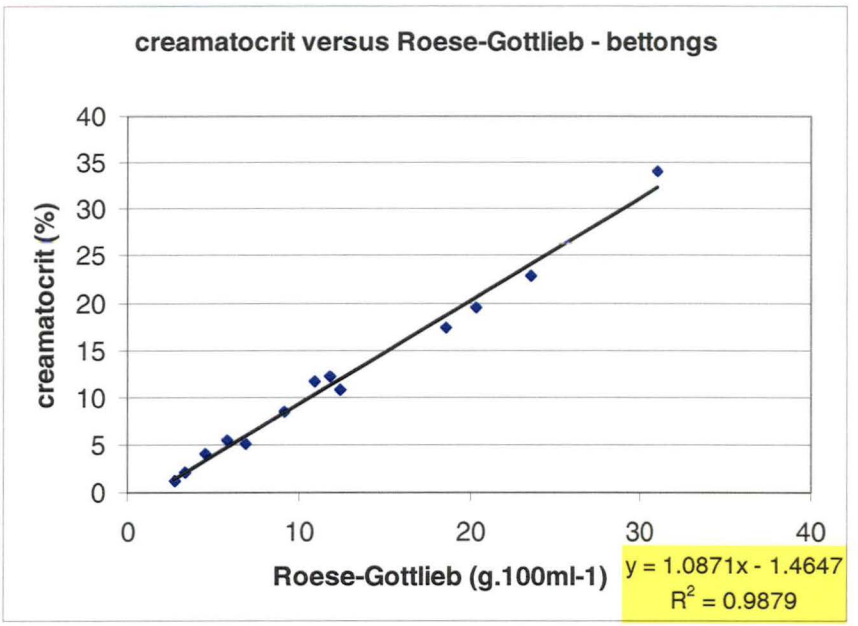

Fig.A.6.3: Linear regression of creamatocrit method and Roese-Gottlieb ether extraction technique for Tasmanian bettong milk samples, N=13.

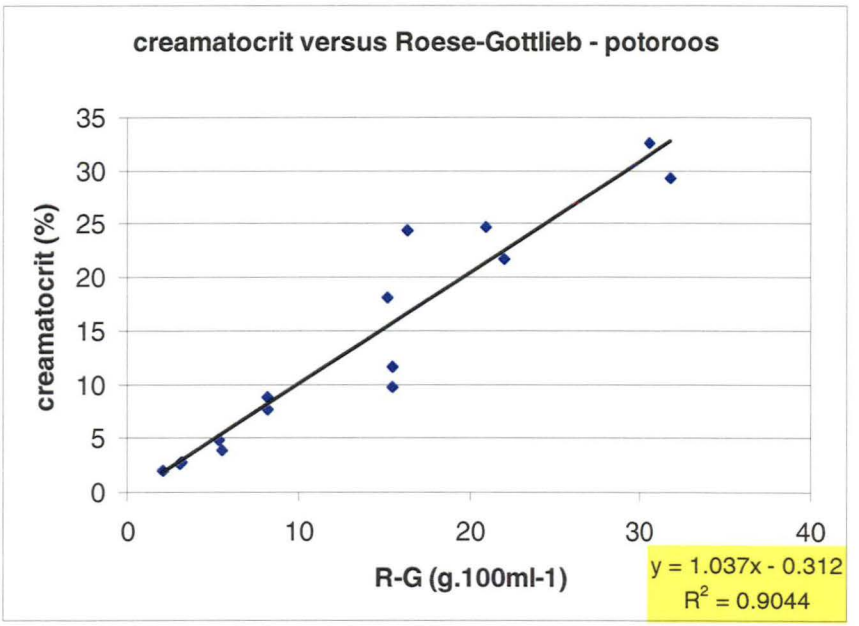

Fig.A.6.4: Linear regression of creamatocrit method and Roese-Gottlieb ether extraction technique for Long-nosed potoroo milk samples, N=14.

A.7 Camera specifications

Weather Proof B/W Bullet Camera (X POSE, Cat. QC-3464)

Pick up element:	1/3" SONY CCD image sensor
Number of pixel:	512(H) x 492(V)<EIA>/512(H) x 582(V)<CCIR>
Resolution:	400 TV lines
Min Illumination:	0.1Lux/F2.0
S/N Ratio:	more than 48dB (AGC off)
Electronic Shutter:	1/60(1/50) to 1/100000sec.
Board Lens:	f3.6mm/F2.0, Angle: 92°
Video Output:	1Vpp, 75ohms
Power Source:	DC12V±10%
Current Consumption:	120mA
Dimensions:	74(L) x 21.5(Dia.)

A.8 Configuration review (actor: mother)

Settings

Setting	Value
Recording method	Continuous
Duration of Observation	Maximum Duration: 00:30:00
Observation timing based on	Elapsed Time

Independent Variables (*Number of Independent Variables: 8)*

Independent Variable Name	Type Values
focal animal	Nominal
species	bettong/potoroo
sex	male/female
category	mother/young
pouch age (pw)	13w – 36w
age	13w – 36w or adult
transfer type	orig/F/CF/NA
general age difference	same/younger/older

Subjects *(Number of Subjects: 7)*

Subject Name
Missing subject
mother
young
other young
other group member
neighbour
vermin

Behaviors *(Number of behavioral classes: 2)*

Behavioral Class 1: individual./social behaviour, Type: Nominal
Number of Elements: 25

Behavior Name	Code	Type	Modifier Class 1	Modifier Class 2
resting	re	State	(None)	(None)
locomotion	lo	State	**type**	(None)
vigilance	vi	State	(None)	(None)
feeding	fe	State	(None)	(None)
drinking water	dr	State	(None)	(None)
investigation	in	State	**form**	(None)
auto-grooming	au	State	(None)	(None)
over-balancing	ov	Event	(None)	(None)
other individual beh.	aa	State	(None)	(None)
actor out of sight	ac	State	**AS**	(None)
approaching	ap	Event	(None)	(None)
distancing	di	Event	(None)	(None)
following	fo	Event	(None)	(None)
sniffing	sn	Event	(None)	(None)
copying	co	Event	(None)	(None)
take food away	ta	Event	(None)	(None)
social encounter	so	State	(None)	(None)
allo-grooming	al	State	(None)	(None)
pouch related	po	State	(None)	(None)
interaction with	ad	State	**Subjects**	**kind**
approach other	af	Event	**Subjects**	(None)
distance other	ah	Event	**Subjects**	(None)
follow other	ai	Event	**Subjects**	(None)
agonistic	ag	State	**ago**	(None)
sexual	se	State	**sex**	(None)

Behavioral Class 2: distance, Type: Nominal
Number of Elements: 16, yin = young in nest

Behavior Name	Code	Type	Modifier Class 1	Modifier Class 2
Body contact	0	State	(None)	(None)
10cm	1	State	(None)	(None)
50cm	2	State	(None)	(None)
1m	3	State	(None)	(None)
2m	4	State	(None)	(None)
3m	5	State	(None)	(None)
4m	6	State	(None)	(None)
distance unclear	8	State	(None)	(None)
KK yin	ab	State	(None)	(None)
10cm yin	ae	State	(None)	(None)

(Behaviour Class 2 continued)

Behavior Name	Code	Type	Modifier Class 1	Modifier Class 2
50cm yin	aj	State	(None)	(None)
1m yin	ak	State	(None)	(None)
2m yin	am	State	(None)	(None)
3m yin	an	State	(None)	(None)
4m yin	ao	State	(None)	(None)
dist. unclear yin	aq	State	(None)	(None)

Modifiers *(Number of modifier classes: 6)*

Modifier Class 1: kind, Type: Nominal
Number of Elements: 5

Modifier Name	Code
Missing kind	?
social	b
agonistic	a
sexual	s
avoiding	c

Modifier Class 2: type, Type: Nominal
Number of Elements: 7

Modifier Name	Code
Missing type	?
2ped	2
4ped	4
stereotypic	s
foray	f
climbing	c
other locomotion	l

Modifier Class 3: form, Type: Nominal
Number of Elements: 4

Modifier Name	Code
Missing form	?
other investigating	i
digging	d
nest building	n

Modifier Class 4: ago, Type: Nominal
Number of Elements: 6

Modifier Name	Code
Missing ago	?
threatening	t
attacking	a
avoiding	b
defending	d
other agonistic beh.	c

Modifier Class 5: sex, Type: Nominal
Number of Elements: 5

Modifier Name	Code
Missing sex	?
investigation	i
mating attempt	m
copulation	c
other sexual beh.	s

Modifier Class 6: out of sight (AS), Type: Nominal
Number of Elements: 4

Modifier Name	Code
Missing AS	?
shelter	s
nest	n
other AS	A

Channels *(Number of channels: 2)*

Channel Name
mother*indiv./soc. BH
mother*distance

A.9 Sociability

Fig.A.6.5: Cross-foster mother-young pair (bettong mother: left, potoroo young: right) resting during the day. Bettong mothers showed high levels of sociability towards their young, but were kept isolated from other bettong adults due to aggressive behaviour, which would have interfered with the study of mother-young behaviour.

Fig.A.6.6: Potoroo group with cross-foster bettong young resting during the day. The grey line on the male's back (left individual) is a result of fur clipping for the behaviour part of this thesis.

Fig.A.6.7: On rare occasions bettongs would form a group in the nest. This, however, was mostly restricted to mother and offspring (young-at-foot: left, mother: middle, subadult daughter: right), but was also observed between two young adults (male and female, unrelated) on a cold day in winter.

A.10 Rehabilitation and release

The Animal Ethics Committee and the Nature Conservation Branch (Department of Primary Industries, Water and Environment) agreed on the rehabilitation and release of surplus animals from the captive colony. A total of 57 animals were rehabilitated and released in conjunction with the wildlife carers network during 2002. Duration of the rehabilitation period varied due to individual circumstances. In general the animals had to display species-specific behaviour (e.g. foraging, nest building, social interactions) before being granted access to the wild. Special care was taken to prevent animals from developing a dependence on the food provided. Rehabilitated females with large pouch young were allowed to stay in the safety of the pen for rearing their young. Animals with injuries or diseases received full veterinary treatment before being released. None of the released animals were wearing radio collars due to financial and time constraints as well as safety concerns. Wildlife carers have sighted released animals on a regular basis (up to the thesis submission date) and reported several offspring, which were fathered in the wild.

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Sterneberg, B.; Rose, R., 2002: Cross-fostering for marsupial conservation. Advances In Ethology. 37: 75